A STUDY OF AUTONOMIC DYSFUNCTIONS AND INTERLEUKIN-6 MEDIATED CHANGES IN SERUM LIPID PROFILE AS BIO-MARKER OF DEPRESSION IN ATTEMPTED SUICIDE PATIENTS

Dissertation submitted to

The Tamil Nadu Dr. MGR Medical University

In partial fulfilment of the regulations for the award of the degree of

M.D. PHYSIOLOGY

Branch V

INSTITUTE OF PHYSIOLOGY & EXPERIMENTAL MEDICINE

Madras Medical College and Rajiv Gandhi Government General Hospital

CHENNAI –600003

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

CHENNAI –600032

MAY 2018

CERTIFICATE

This is to certify that the dissertation entitled **"A STUDY OF AUTONOMIC DYSFUNCTIONS AND INTERLEUKIN-6 MEDIATED CHANGES IN SERUM LIPID PROFILE AS BIO-MARKER OF DEPRESSION IN ATTEMPTED SUICIDE PATIENTS"** by the candidate **Dr. K.SOWMIYA** for M.D Physiology is a bonafide record of the research done by her during the period of study (2015 –2018) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai –600003.

DEAN
Madras Medical College
Chennai

Director and Professor
Institute of Physiology and
Experimental Medicine,
Madras Medical College,
Chennai-600003

GUIDE

CANDIDATE

ACKNOWLEDGEMENT

There is hardly any task than acknowledging my gratitude to all those who have helped me in so many ways during my course.

I gratefully and sincerely thank **Dr.R.NARAYANA BABU**, M.D., DCH. the Dean of Government Madras Medical College and Hospital, Chennai for granting me permission to carry out the study at the Institute of Physiology and Experimental Medicine, Madras Medical College and Hospital.

I will forever be thankful to **Prof. Dr.A.SHAKEELA BANU ,M.D.,** the Director and Head of Department of Physiology, Madras Medical College, Chennai for providing insightful discussions about the research and giving me the opportunity to develop my own individuality and allowing me to work with such independence.

I am thankful to **Prof. Dr.K.SRINIVASAGALU, M.D**, Former Director, Institute of Internal Medicine , Rajiv Gandhi Government General Hospital, Chennai, for granting me permission to recruit cases from the Department.

I am extremely grateful to my guide **Prof. Dr. R. VIJAYALAKSHMI, M.D.,** for her consistent support throughout my study period and her valuable suggestions to improvise my work at all stages.

I extend my sincere thanks to **Prof. Dr.C.THIRUPATHI, M.D., D.C.H.,** Professor, Institute of Physiology, Madras Medical College, Chennai, for his valuable suggestions and motivation throughout my study.

I extend my sincere thanks to **Prof. Dr.P.SATHYA, M.D., D.G.O.,** Professor, Institute of Physiology, Madras Medical College, Chennai, for her valuable suggestions and motivation throughout my study.

I extend my sincere thanks to **Prof. Dr.A.PARIMALA, M.D., D.C.P.,** Professor, Institute of Physiology, Madras Medical College, Chennai, for his valuable suggestions and motivation throughout my study.

I extend my thanks to **Prof.Dr.RAMA DEVI, M.D.,** Professor and Director, Institute of Biochemistry for her kind permission to do the lab test in their department.

I would also like to express my gratitude to **Prof. Dr. PUSHKALA, M.D.,** Professor and HOD, Department of Immunology, TN Dr. MGR Medical University for permitting me to analyse my samples in the department laboratory.

I extend my sincere thanks to **Dr.J.Ratna Manjushree, M.D., D.C.H.,** Associate Professor, Institute of Physiology, Madras Medical College, Chennai, for her valuable suggestions and motivation throughout my study.

I express my sincere thanks to **Dr.T.N.Vijayalakshmi,M.D., Dr.Shanthimalar,M.D., Dr.S.Kavitha,M.D., Dr.K.Aanandha Subramanium,M.D., Dr.V.Gowri,M.D., Dr.Indhumathi.D, M.D., Dr.syed safina,M.D., Dr.Anitha Ponmalar,M.D., Dr.V.Sumathi M.D.,**Assistant Professors ,Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai for their guidance and support.

I express my sincere thanks to my Colleagues in department of Physiology, Madras Medical College, Chennai and my dear friends who readily extended their help to overcome the difficulties of my task.

I thank all the technical and non technical staff of IPEM, Institute of Internal Medicine, Institute of Biochemistry, Madras Medical College and Department of Immunology, TN Dr. MGR Medical University for their timely help to complete my study.

Above all I would be unfair if I fail to mention my special gratitude to my dear parents, my lovable husband and my precious children, who are the pillars of my career and without whom it would have been impossible to accomplish this work.

I dedicate this work to my supportive family.

Finally I thank Almighty for keeping me blessed always in all my endeavours.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

LIST OF PHOTOGRAPHS

ABBREVIATIONS

5-HT	5- Hydroxy Tryptamine
ANS	Autonomic Nervous System
BBB	Blood Brain Barrier
BDI	Beck's Depression Inventory
BDI PC	Beck's Depression Inventory Primary Care
BDNF	Brain Derived Neurotropic Factor
CIDI	Composite international diagnostic interview
CMD	Common mental disorders
CNS	Central nervous system
CRH	Corticotropin releasing hormone
CRP	C- reactive protein
CSF	Cerebro spinal fluid
DALY	Disability adjusted life years
DIS	Diagnostic interview schedule
DSM	Diagnostic and statistical manual
ECG	Electro cardio gram
ELISA	Enzyme linked immune sorbent assay
HDL	High density lipoprotein
HF	High Frequency
HPA	Hypothalamo pituitary adrenal axis
HRV	Heart rate variability
ICD	International statistical Classification of Diseases
ICPE	International Consortium of Psychiatric Epidemiology
IFN γ	Interferon gamma
IL 6	Interleukin 6
LDL	Low density cholesterol
LF	Low frequency
LPS	Lipopolysaccharide

MAD	Major affective disorder
MDD	Major depressive disorder
MDE	Major depressive episode
NCRB	National crime records buereau
NE	Norepinephrine
NST	Nucleus of Tractus Solitarius
NT-3	Neurotropin 3
POMC	Pro opiomelanocortin
RSA	Respiratory sinus arrhythmia
SLE	Systemic lupus erythematosus
SUD	Substance use disorders
TC	Total cholesterol
TGL	Triglycerides
TNF α	Tumour necrosis factor alpha
TRD	Treatment resistant depression
VLDL	Very low density lipoproteins
VLF	Very low frequency
WHO	World health organization
WMH	World mental health survey
YLD	Years Lived with Disability

CERTIFICATE – II

This is to certify that this dissertation work titled **A STUDY OF AUTONOMIC DYSFUNCTIONS AND INTERLEUKIN-6 MEDIATED CHANGES IN SERUM LIPID PROFILE AS BIO-MARKER OF DEPRESSION IN ATTEMPTED SUICIDE PATIENTS** of the candidate **Dr.K.SOWMIYA** with registration Number **201515003** for the award of **M.D Degree** in the branch of **PHYSIOLOGY**.

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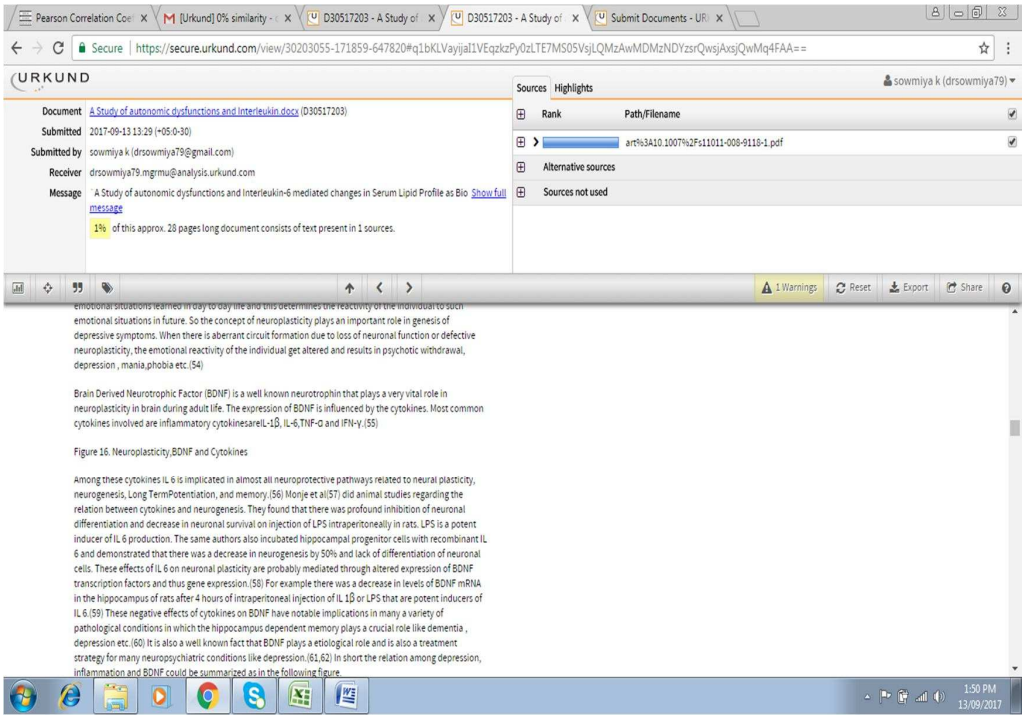


Introduction

INTRODUCTION

ATTEMPTED SUICIDE:

"An act with non-fatal outcome, in which an individual deliberately initiates a non-habitual behaviour that, without intervention from others, will cause self-harm, or deliberately ingests a substance in excess of the prescribed or generally recognised therapeutic dosage, and which is aimed at realising changes which the subject desired via the actual or expected physical consequences".[1]

These behaviors may be carried out after meticulous preplanning or may be impulsive. The motive varies between simple attention seeking to real intention to die. Sometimes these behaviors are thought to be carried out in order to threaten the family members or to divert their attention during crisis.

Whatever may be the reason for attempting , such a behavior indicates that the individual involved is in distress. The cause for distress widely varies. Common causes for attempting suicide depends upon the age, gender, ethinicity, socio-economic status , education, social support etc.

Family history	Suicidal behaviour, mental disorders
Mental disorders	Any disorder, depression, substance use disorders, personality disorders, schizophrenia
Contact with psychiatric services	Any contacts, recent contacts, post-discharge period, psychotropic drugs
Psychiatric symptoms	Hopeless, helpless, depressive, psychotic, delirious, anxious, aggressive, introversive
Suicidal behaviour	Previous suicide attempts, suicidal ideations, death wishes, indirect gestures
Physical health	Severe physical illness such as cancer, AIDS, stroke, and epilepsy; permanent sickness
Availability of suicide methods	Easy access to lethal methods

Figure 1. Clinical Determinants of Suicide

Neurobiology of attempted suicide:

Many theories were proposed for a better understanding of suicidal behavior. The stress diathesis model of suicide outlines the link between the predisposing factors and precipitating factors.

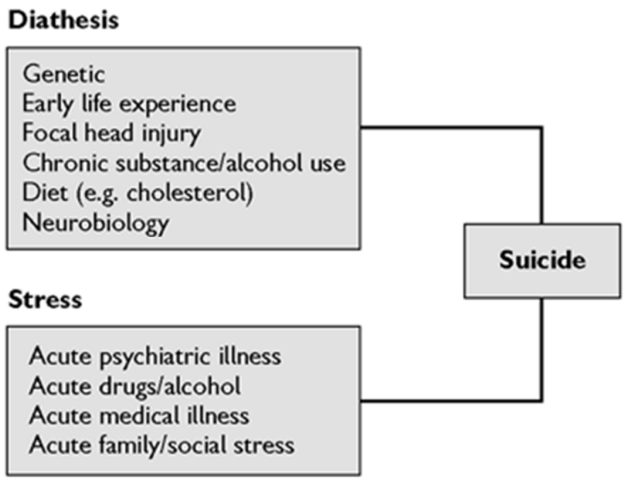


Figure 2. Stress Diathesis model of Suicide

Mann et al[2] were the first to propose the Stress-Diathesis model of suicide and they outlined the following pathway as the course towards suicide.

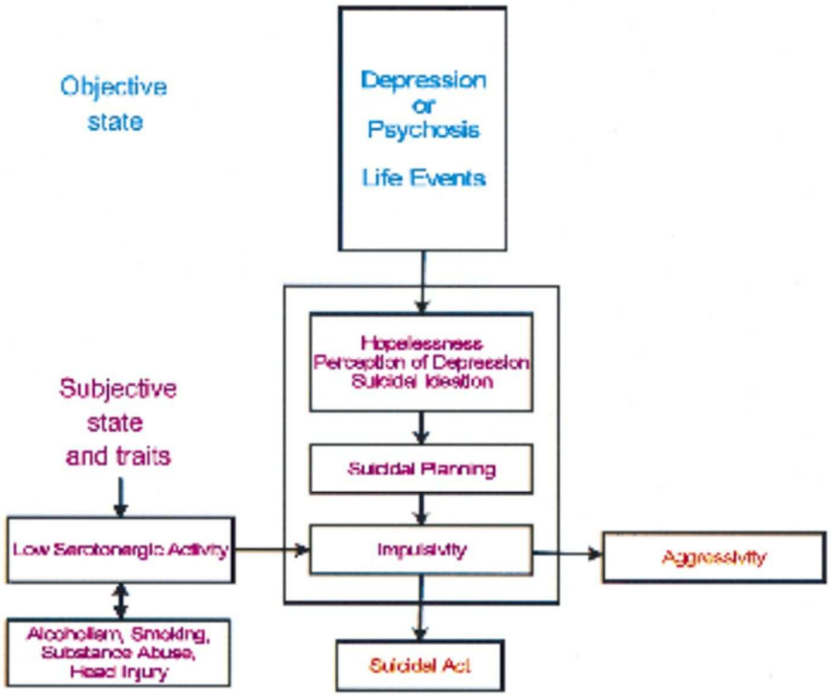


Figure 3. Pathway Leading to Suicide in Stress Diathesis Model

Among psychiatric illness suicide is one diagnosis which is more related to the mind than the brain. But researchers have been constantly working on finding out a biological cause for suicide so that prevention strategies could be formulated.

Low levels of serotonin in the brain and the dysfunction of the noradrenergic system has been found to be the cause for impulsiveness and aggression which leads to suicidality. When cause for low serotonin levels were

probed low cholesterol and altered cytokine levels in the circulation were found to present in these set of patients.

Also the same biological picture was found in patients with depression who attempt suicide.

DEPRESSION :

Depression is a very complex clinical construction. It can affect anyone irrespective of race, gender, age. The chances of developing depression is 1 in 5 for women and 1in 10 for men. Lifetime prevalence of depression in Indians exceed 30% . Depression is one end of the spectrum of mood disorders, the other being mania. The table below outlines manic-depressive spectrum.[3]

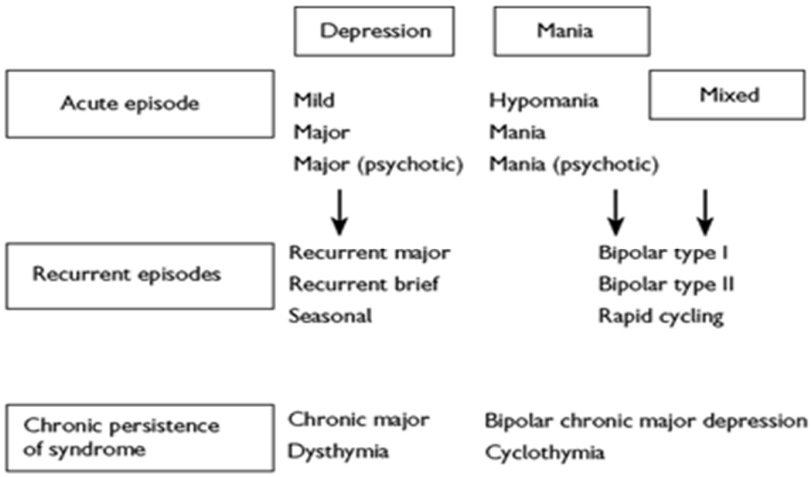


Figure 4. Spectrum of Mood Disorders

Depression presents with varied symptoms of which the essential symptoms are depressed mood and anhedonia.

	Symptoms of depression	DSM-IV	ICD-10
1	Depressed mood most of the day, nearly every day	+	+
2	Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day	+	+
3	Loss of energy or fatigue nearly every day	+	+
4	Loss of confidence or self-esteem		+
5	Unreasonable feelings of self-reproach or excessive or inappropriate guilt, nearly every day	+	+
6	Recurrent thoughts of death or suicide, or any suicidal behaviour	+	+
7	Diminished ability to think or concentrate, or indecisiveness, nearly every day	+	+
8	Psychomotor agitation or retardation nearly every day	+	+
9	Insomnia or hypersomnia nearly every day	+	+
10	Change in appetite (decrease or increase with corresponding weight change)	+	+

+ indicates that the symptom is included.

Figure 5. Comparison of ICD-10 and DSM IV symptoms of Acute Depression

Depression causes distress not only to the individual affected but also to their families and the society. It is a great economic burden to the society.

Neurobiology of Depression:[4]

Depression is nowadays considered as a defect of biological phenomenon. Research is being focused on finding the defect in the body that leads to the development of depression in vulnerable individuals. Psychological stress and genetic vulnerability were regarded as the causative factors for a very long time. Newer hypothesis concentrate on the pathology in the monaminergic pathways &

HPA axis dysregulation. These hypotheses provide a promising direction in developing an acceptable and effective treatment protocol for depression.

The role of cytokines is causing depression is the area of interest in recent times.In fact depression is found to be a chronic inflammatory process. The association of depression with diseases like diabetes, cancers , rheumatoid arthritis, cardiac conditions etc. raises a suspicion that depression shares a common etiology with theses chronic inflammatory diseases.

Though the psychological stress caused by these chronic diseases can be attributed to the causation of depression, the link between the proinflammatory cytokines that are present in an elevated state in these diseases and the tryptophan –serotonin metabolism has been postulated and proves to be a cause for genesis of depression in such conditions.

CYTOKINES:

Cytokines are chemical messengers in the body that act in auto-para-endocrine fashion. They are secreted in very small quantities in nanomolar or picomolar concentrations but exert very huge effect on both physiology and pathology of the human body.

They play a crucial role in survival, growth, differentiation and effector functions of the cells. Also they are the key regulators of immune response occurring in many pathological conditions involving the joints, kidney, brain and endocrine system.

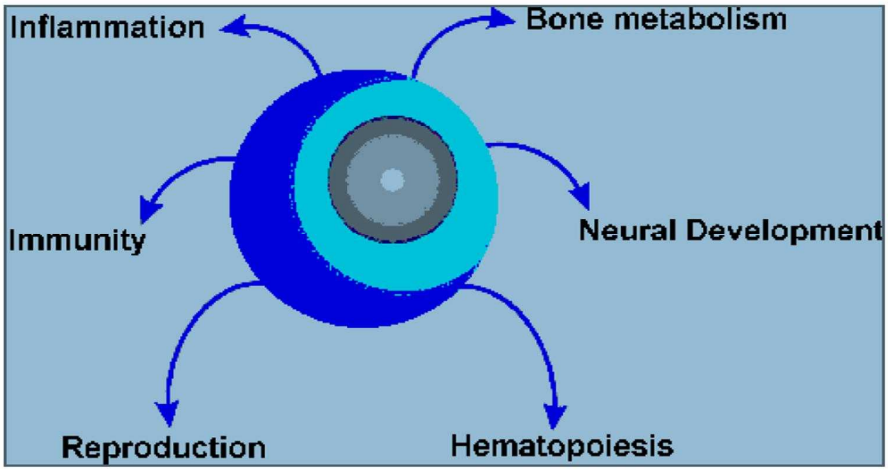


Figure 6. Cytokines in Physiological processes

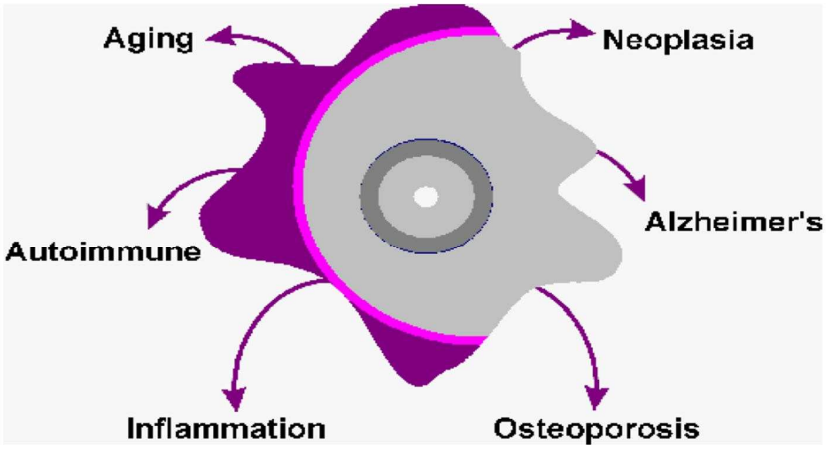


Figure 7. Cytokines in Pathological processes

Unlike hormones they are not constantly produced or stored in the body but rapid synthesis of cytokines occur in response to stimuli like stress and infections. Also it is very difficult to detect them in circulation as the secreting cells are usually very close to the target cells. So detection of cytokines in peripheral

circulation indicates abnormal synthesis which mostly occurs in underlying stress-both somatic and psychic. Interleukin 6 is one such cytokine.[5]

INTERLEUKIN-6:

IL-6 is both a pro-inflammatory and anti-inflammatory cytokine. It is found to be expressed in almost all cell types in the body and is species specific. IL 6 is secreted by the monocyte/macrophages, endothelial cells and fibroblasts during a systemic inflammation. It behaves as an acute phase reactant, the main target cells being the hepatocytes.[6]

Physiological actions of IL 6:

Acute phase response through production of variety of hepatic proteins-CRP, amyloid A, fibrinogen etc[7]. B cell differentiation that leads tp immunoglobulin production. [8] Proliferation of both thymic and peripheral T cells and their differentiation to cytolytic and natural killer cells[9] Bone remodeling through activation of osteoclastogenesis[10] differentiaion and proliferation of neural cells.[11]

During a local inflammation of the CNS and joint spaces, IL 6 can be detected in the CSF and synovial fluid[12,13].

This shows that astroglia, microglia and synoviocytes may also be the source of IL 6 production. The other cytokines like IL 1 and TNF-α also exert their actions through IL 6 by inducing its secretion by these cells. Normally IL 6

levels are undetectable or it is found in trace amounts say < 1pg/ml. During inflammatory processes the levels increase even upto thousand fold.

Thus the actions of IL 6 are pleiotropic.

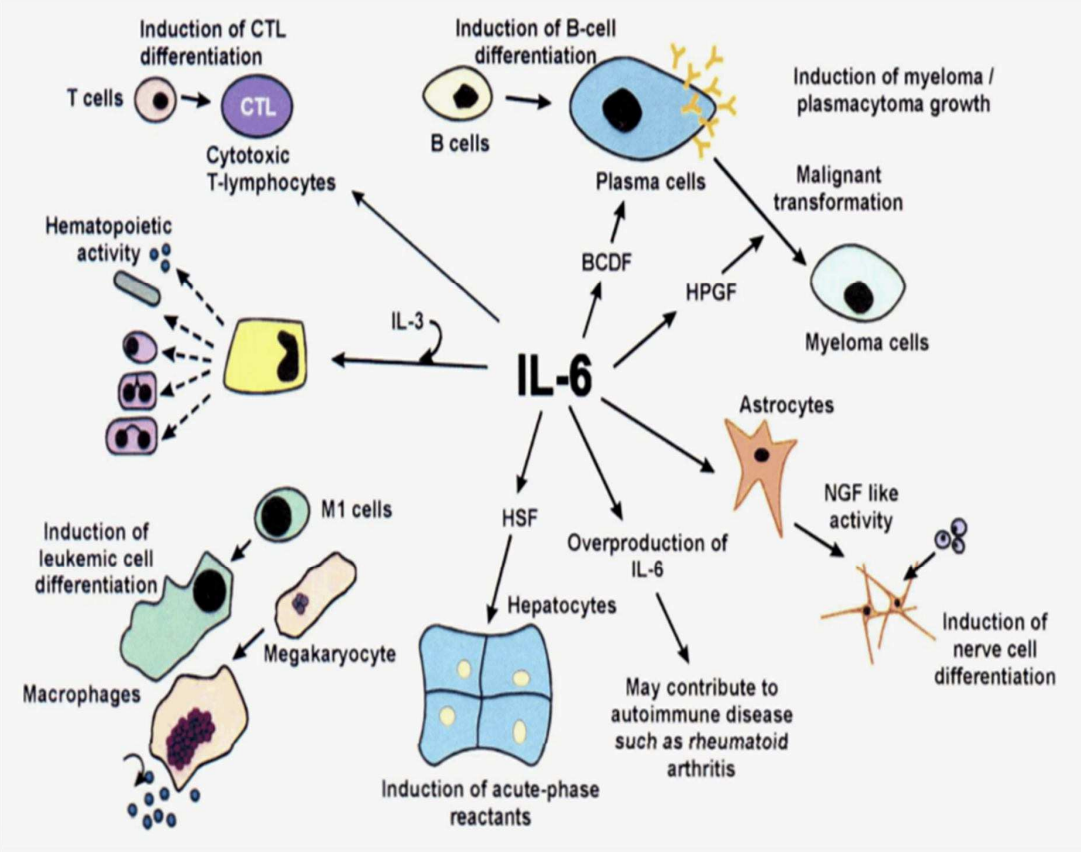


Figure 8. Pleiotropic Actions of Interleukin 6

Interleukin 6 also plays a role in chronic inflammation. It evokes the acute phase responses in inflammation through stimulation of Hepatocytes. IL 6 also acts upon the HPA axis and elevates the Serum Cortisol levels.

Under normal physiological conditions glucocorticoids suppress IL 6 production. So when there is a surge in IL 6 levels during stress and inflammation

a negative feedback loop is established and stimulation of CRH occurs which activates the HPA axis.[14,15]

Activation of HPA axis is one of the neurobiological process that is being postulated in the genesis of depressive states. The overproduction of IL 6 has been documented in chronic inflammatory diseases like Rheumatoid arthritis, SLE, Psoriasis, Diabetes etc. Chronically elevated levels are seen in persons with increased risk for cardiovascular morbidity. High IL 6 also induces malignant transformation of B-Lymphocyte lineage. All the above mentioned conditions are found to be associated with Depression. So IL 6 may be the common underlying factor for the development of Depression.

CHOLESTEROL- ROLE IN CNS[16]

Lipids are one of the important constituents of the body. Lipids of major physiologic significance are fatty acids and esters, cholesterol and steroids. Eicosanoids are a group of substances like Prostaglandins, Leukotrienes etc. are physiologically lipids. Lipoproteins are precursors of second messenger system. Glycerophospholipids and Sphingolipids are major constituents of membranes and CNS. Glycolipids are found in nervous tissue especially brain.

Cholesterol is found in many tissues of the body including membranes and it is the parent molecule of many physiologically important substances like steroid hormones, bile acids etc.

Of all these forms of lipids Cholesterol is especially found in Central Nervous System about 35 gms in adult brain [17]. In fact brain is the cholesterol rich organ in the body lodging about 20 % of total body cholesterol. [18]

Cholesterol in the brain is responsible for the fluidity of membranes, membrane permeability and exchange of substances. Cholesterol is also found to play a role in synaptogenesis and dendrite formation. Since they are constituents of neuronal membrane, they are very much essential for neurotransmission.[19]

The effects of lipids on membrane fluidity modulates the binding of serotonin to 5-HT receptors. Low cholesterol increases reuptake of Serotonin at presynaptic end and decreases serotonin receptor number and function in postsynaptic sites.[20]

Thus Low cholesterol availability to brain causes alterations in serotonin function which is the major neurotransmitter involved in mood disorders especially depression.

HEART RATE VARIABILITY – A MEASURE OF AUTONOMIC FUNCTION:

Heart Rate Variability (HRV) is a measure of beat-to-beat variability in continuous recording of ECG. It can be either variation in heart rate or variations in the R-R interval. Reduced HRV is a negative prognostic indicator for a wide range of diseases and high robust variations in heart rate is an indicator of good health.

Since the variations were found to be in accordance with the respiratory cycle it is also termed as Respiratory Sinus Arrythmia (RSA).

The physiological basis of HRV is a subject of investigation and still is controversial. Traube et al. (1865) proposed that the central medullary neurons regulating the respiration irradiated the cardiac centres also and affect the arterial pressure waves. Karl Eward Hering (1871) made a conclusive statement that the reflex activation of the afferent fibres to the lungs is the cause for these periodic changes.

Bainbridge (1930) proposed that the variability is due to the mechanical distortion of atria due to intrathoracic pressure changes according to the respiratory cycle and not due to neural involvement. Franciscus C. Donders (1868) came out with a suggestion that the changes in heart period in association with respiration is a result of vagal activation.

The contribution of sympathetic nerve stimulation to HRV was proved by Guyton and Harris (1951). A multitude of complex experiments were performed in mammals like dogs and cats including humans over nearly 40 years.

Today it is widely accepted that HRV at any point of time is a measure of complex interactions between parasympathetic and sympathetic nerve fibres on the sinoatrial node along with the mechanical factors acting upon the heart as a result of respiratory cycle.

Thus HRV reflects the sympatho-parasympathetic balance in the body at any point of time.[21]

Analysis of HRV[22]

Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology formulated the guidelines and Standards of measurement, physiological interpretation, and clinical use. The guidelines provided two broad domains of measures to standardize physiological and clinical research.

1. Time domain methods
2. Frequency domain methods

HRV can be a short term analysis for 5 minutes or a long term recording for 24 hours. Frequency domain analysis is used for short term recording and Time domain analysis is done for long term recording.

Time domain methods:

Time domain measures are easier to calculate but provide less information. A continuous ECG is recorded and the heart rate is calculated at a given point of time. Successive normal QRS complexes are identified and the interval between them is determined.

This is called NN interval ie. Normal to normal interval. This data is subjected to statistical or geometric analysis. In practice, the NN interval equals the RR interval.

The time domain metrics are statistical methods. To make them significant time domain measures should be calculated over a specific and fixed period of time or epoch. SDNN reflects the actual variability and HRV gets reduced according to this value. Mean HR is the Mean of the selected RR interval and Mean RR is the Mean of the selected RR series.

Variable	Units	Definition
TIME DOMAIN MEASURES		
a. Statistical		
SDNN	ms	SD of all normal R–R intervals
SDANN	ms	SD of the average normal R–R intervals calculated over short time periods (usually 5 min) for the entire recording period (usually 24 h)
RMSSD	ms	The square root of the mean squared differences between adjacent normal R–R intervals
SDNN index	ms	Mean of the SD of the normal R–R intervals calculated over short periods time (usually 5 min) for the entire recording period (usually 24 h)
NN50		The number of pairs of adjacent normal R–R intervals that differ by more than 50 ms
pNN50	%	NN50 divided by the total number of normal R–R intervals × 100
b. Geometrical		
HRV triangular index		Number of normal R–R intervals divided by the height of the histogram of all the normal R–R intervals measured on discrete scale with bins of 1/128 s (7.8125 ms)
TINN	ms	Baseline width of the minimum square difference of triangular interpolation of the highest peak of the histogram of all normal R–R intervals
FREQUENCY DOMAIN MEASURES		
Total	ms^2	Area under the entire power spectral curve (usually ≤0.40), variance of all normal R–R intervals
ULF	ms^2	Ultra low frequency power (≤0.003 Hz)
VLF	ms^2	Very low frequency power (0.003–0.0.04 Hz)
LF	ms^2	Low frequency power (0.04–0.15 Hz)
HF	ms^2	High Frequency power (usually 0.15–0.40 Hz*)
LFnu	nu	Normalized low frequency power (LF/LF + HF)
HFnu	nu	Normalized high frequency power (HF/LF + HF)
LF/HF		Ratio of the low-to high frequency power

*Nu, normalized units; *HF is shifted to higher ranges (0.24–1.04 Hz) in infants and exercising adults.*

Figure 9. Conventional HRV measurements

Frequency domain methods:

Analysis of a ECG record using a set of mathematical operations gives the frequeny domain methods. A series of R-R intervals are chosen and processed through parametric and non parametric methods.(marple, 1987). Methods used are

Autoregression and Fast Fourier Transformation. These methods analyse the frequency information by breaking them down into smaller oscillations. Calculations are done for the same epoch duration as for SDNN and the results are expressed in terms of spectrum of power. This Power Spectral density (PSD) analysis gives a picture of the power distributed as a function of frequency. Analysis is done by calculating power and peak frequencies in different bands.

In a short term ECG record three main frequency bands are spectrally analysed as follows.

High frequency power (HF, 0.15-0.4Hz):

This is a measure of parasympathetic activity. Reflects the activity of Vagus nerve under the influence of modulations in gas exchange and RSA. Could be blocked parasympatholytics. Can be done even with a 1 min record.

Low frequency power (LF, 0.04-0.15Hz):

Measure of the sympathetic nervous modulation. Indirect measure of the baroreceptor reflex mechanisms. A minimum of 2 minute ECG record is needed.

Very low frequency power (VLF, 0-0.04Hz):

Not much dependable measure in a short term record because an acceptable explanation of its physiological occurrence could not be derived.

Normalization of units:

LF (n.u) and HF (n.u) are the normalization of the powers which gives near 100% values of the sympathetic and parsympathetic events. They are calculated as follows:

LF (n.u) = LF power/(LF + HF power) or LF power / TP-VLF

HF (n.u) = HF power/ (LF+HF power) or HF power / TP-VLF

Total power = LF+HF power

LF/HF Ratio:

Ratio is calculated after normalization of the LF and HF.(Mallinai A et al, 1991)[23]. Since the distribution of LF and HF varies according to the autonomic modulation of heart rate this is regarded as an index of sympatho-vagal balance.

Review of literature

REVIEW OF LITERATURE

Epidemiology of attempted suicide:

The epidemiology differs throughout the world. The WHO fact sheet for suicides states that around 800 000 people die of suicide throughout the world and around 20 times more people attempt suicide. On an average one death occurs every 40 seconds and one attempt every 3 seconds. The rates of suicides range from 0.7-58.8/1 lakh throughout the world.

The Indian rates are 16/1 lakh according to the WHO suicide rates in 2015. These were the rates in the age group of 15-44 years considered as the productive age group. Suicide has become the second leading cause of death among 15-29 year olds. And it is the 17th leading cause of death throughout the world.[24]

The Indian Scenario:

Suicide is one of the leading causes of death in India.

The National Mental Health Survey reports on suicidal risk in Indian population is alarming. According to this survey reported 2015-16 nearly 1% of the population is at high suicidal risk. Half of this population suffer from a co-morbid mental illness while the other half do not. So causes for suicide in Indian population needs a quality research at all levels starting from the primary care level.[25]

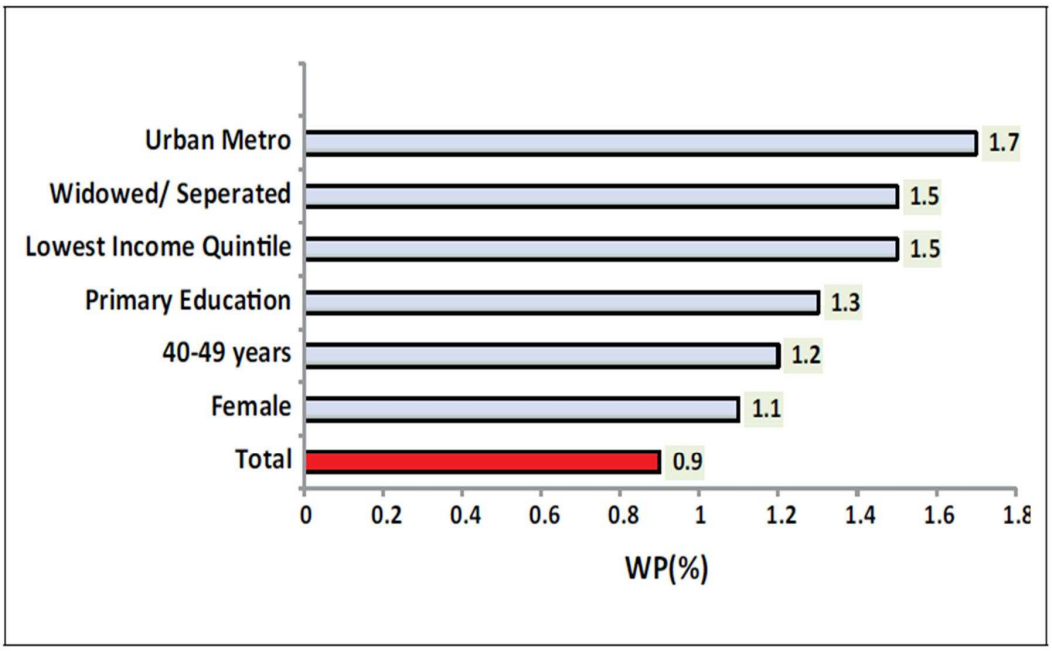


Figure 10. Prevalence of High suicidal Risk

The current NCRB statistics for suicides and attempted suicides in India states that the rate of suicide is 10.6/1 lakh population for the year 2015. But this data compiled from the police records. So it is not the exact figure as, under reporting of suicides and attempts is also suspected due to social stigma and taboo.[26]

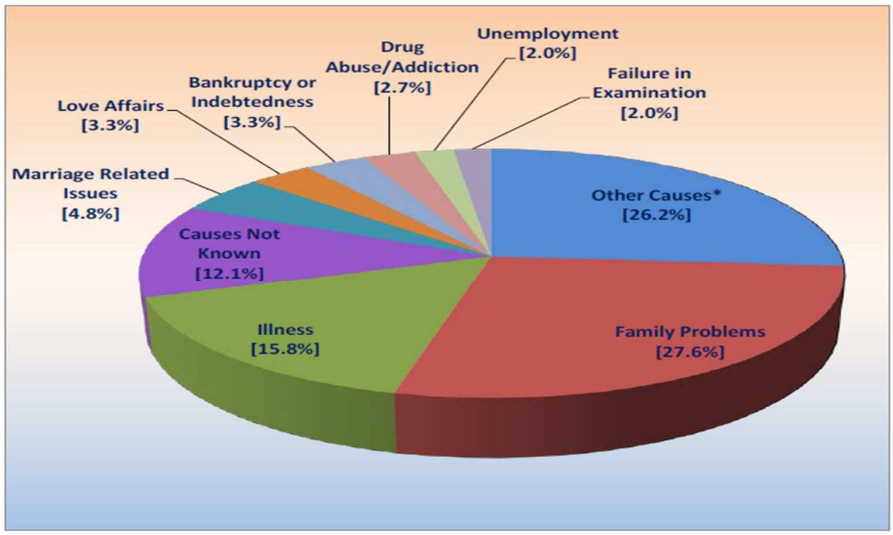
Number of Suicides, Growth of Population and Rate of Suicides during 2011 - 2015

Sl. No.	Year	Total Number of Suicides	Mid-Year Projected Population* (in Lakh)**	Rate of Suicides*** (Col.3/Col.4)
(1)	(2)	(3)	(4)	(5)
1	2011	1,35,585	12,101.9#	11.2
2	2012	1,35,445	12,133.7	11.2
3	2013	1,34,799	12,287.9	11.0
4	2014	1,31,666	12,440.4	10.6
5	2015	1,33,623	12,591.1	10.6

* –Mid-year Projected Population as on 1st July; Source: The Registrar General of India
\# --Population of the Population Census, 2011; Source: The Registrar General of India
** – One Lakh = 0.1 Million
*** – Rate of Suicides = Incidence of suicides per one lakh(1,00,000) of population.

Figure 11. NCRB statistics on suicide (2011-2015)

The common causes for attempting suicide in India is as shown in the below pie chart.



* Figure of Suicides due to Poverty, Unemployment, Physical Abuse, Professional/Career Problem, etc. included along wi
Other Causes.

Figure 12. Causes of Suicide in India

According to the above seen statistics the causes for suicides in India can be ranked in the following order.

Table 1. Causes Of Suicide In India

S.No.	Cause	%
1.	Family Problems	27.6
2.	Other causes	26.2
3.	**Illness**	**15.8**
4.	Causes not known	12.1
5.	Marriage related issues	4.8
6.	Love Affairs	3.3
7.	Bankruptcy & indebtness	3.3
8.	Drug abuse & addiction	2.7
9.	Unemployment	2.0
10.	Failure in examinations	2.0

Mental illness and suicide:

Suicide is considered as highly personal and individual act. But suicidal behaviour is influenced by social , environmental and physiological factors. According to Durkheim " suicide is the outcome of social and societal situations". But Esquirol opposed this theory by writing on suicide as " All those who committed suicide are insane". Still there is a debate whether suicide results from external stressors or due to mental illness.

The data on suicides shows that family problems and illness are equally responsible for suicide. Among the illnesses mental illness is the major causative

factor. Several studies have concluded that about 90% of the suicide cases have a diagnosable mental illness.

In India the two case control studies done in Chennai and Bengaluru show that affective disorders are the most important diagnosis and around 25-35% of them were mood disorders, particularly Depression of varying degrees. Majority of cases attempt suicide even in the first episode of depression.[27]

Psychological autopsy studies in completed as well as attempted suicides show that depression, previous attempts, addiction, antisocial traits, impulsivity and obsessionality are frequently found predisposing factors.

Among those who attempt suicide 10- 15 % tend to repeat attempts in the next 6 months and the rate increases to 20% in the next one year or two. High suicidal intent, depression and substance abuse predict high risk of repeated attempts and completed suicide[28].

Indian studies on suicide:

The psychological autopsies done on suicidal deaths and studies conducted on suicide attempters in various parts of the country give a widely varied results. These may be due to the notable cross cultural variations in the country and differences in the methodology of such studies.[29]

According to Venkoba Rao et al suicides are lesser in Indian depressives when compared to the western world. The authors say that suicides or attempts in

depressive illness are due to guilt feelings, which are not so predominant in the Indian patients.[29,30]

But in a study conducted by A.Badrinarayana[31], in a psychiatric hospital at Karnataka, depressive patients were compared with schizophrenics. Among them a significant positive association was found between depressive illness and suicidal tendencies. Also high scores of hopelessness and helplessness were present in contrast to previous findings that somatic symptoms of depression were the risk factors of suicides.[32]

Another study conducted on 154 suicide ideators in a general hospital of south Tamilnadu around 59.74% of them got a diagnosis of depression according to the ICD-10.[33] The same study shows that on a symptom wise analysis hopelessness and worthlessness showed higher scores in the depressive patients.

Sharma and Jain et al [34,35]concluded in their study that 15-34 yrs is the commonest age group attempting suicide. Females and unmarried formed about 50% of the study group in both the studies. Majority consumed organophosphorous compounds. When the causes were explored quarrels with spouse, in-laws, parents, failure in love were the commonest causes with psychiatric problems being the top in the list.

Epidemiology of depression - Global scenario:

In the Global burden of disease project of the WHO the authors state that the attributable burden of depression in the society is greater as it includes the burden of suicide also.[36]

Weissman et al[37] were the first to publish comparative cross-national study of MDD according to DSM III. This study was conducted in 10 countries where population based surveys were conducted using Diagnostic Interview Schedule (DIS)[38]. The lifetime prevalence of depression was in the range of 1.5% to 19.0% with a mean of 9.2% and the 12 month prevalence was 0.8% to 5.8% with a mean of 3.0%.

The ICPE survey conducted by Andrade et al[39] in 10 different countries with a sample size of 3700 using the Composite International Diagnostic Interview (CIDI) reported a lifetime prevalence of Major Depressive Episodes (MDE) as3% - 16.9% with majority of them falling between 8% to 12%.

Evelyn Bromet et al [40]published a research article on epidemiology of depression according to DSM IV criteria in 18 countries across the world that participated in the World Mental Health Survey WMH initiative. Among these 10 countries were high income countries and remaining 8 were low-middle income countries. The lifetime prevalence was 14.6% and 11.1% in the above mentioned respective group of countries. The 12 month prevalence was 5.5% and 5.9% respectively.

According to the Global Burden Of disease and Injury series[41] published by the WHO in 1990 infectious diseases were the leading cause of death and disability during those periods. The authors of this series tried to calculate disease burden in terms of DALY and this lead to a discovery that non communicable diseases top the list. Depression ranked 4th in 1990. But when the data was projected to 2020 depression moved to the 2nd place.

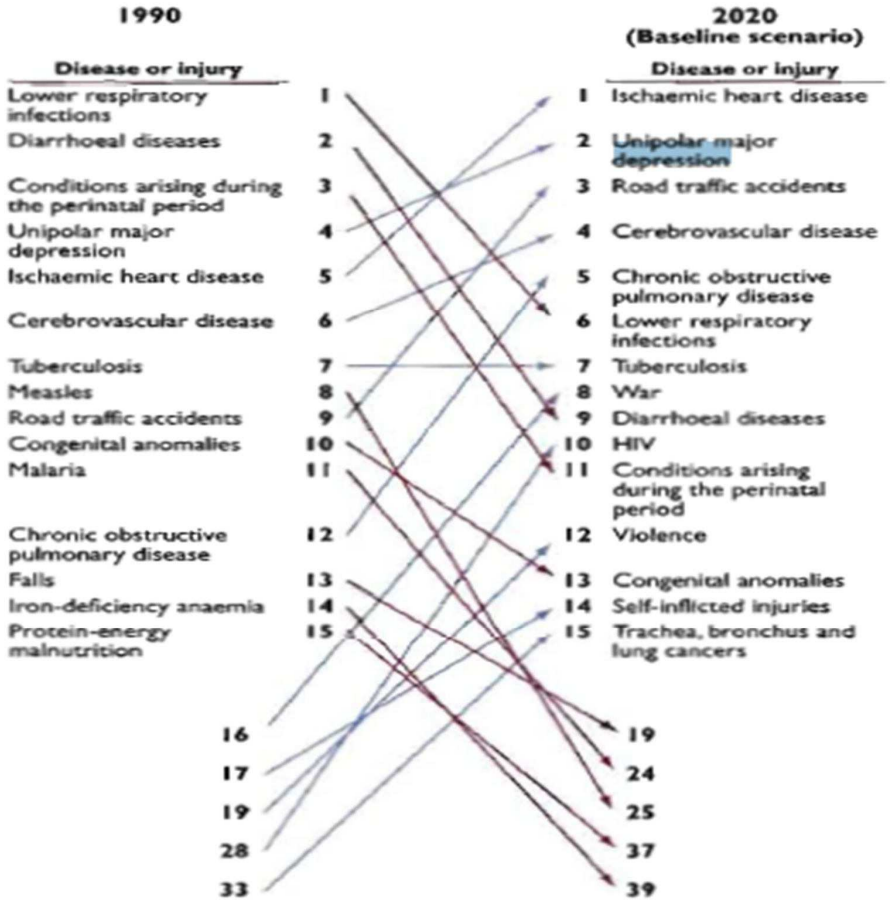


Figure 13. Disease Burden in DALYs

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As predicted now depression is ranked as the single largest cause of disability by the WHO in terms of DALY. According to WHO 7.5% of all years lived with disability is due to depression. The global estimate of depression in 2015 is 300 million people across the world. this equals 4.4% of the world's total population . globally there is a 18% increase in depressed population from 2005 through 2015. [42]

Indian scenario:

According to the National Mental Health Survey[25] statistics for the year 2015-16 depression is the leading cause of disability in terms of DALY and YLD. Anxiety, SUD and Depression are now designated as common mental disorders(CMD) as they are present as comorbid conditions as well as causative factors of chronic and serious medical illness. These CMDs affect nearly 10.0% of the population.

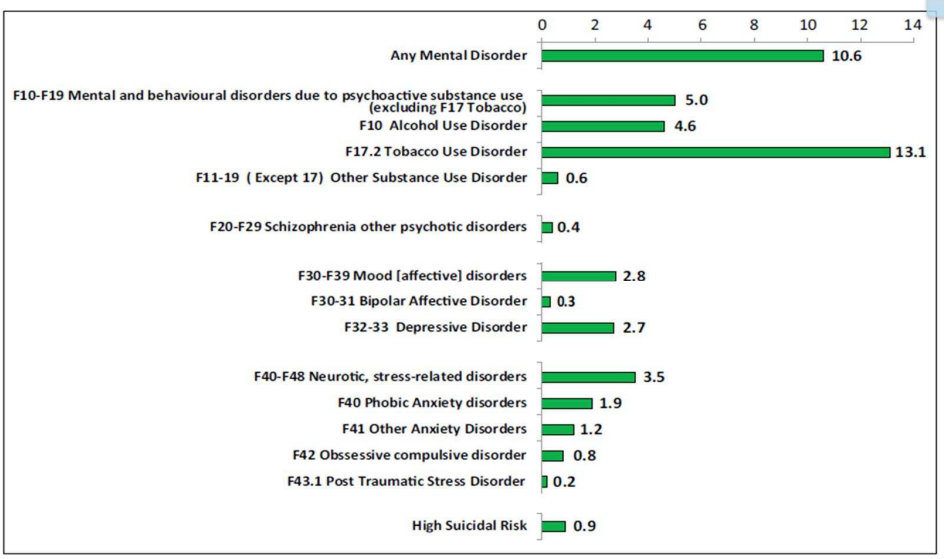


Figure 14. Prevalence of Mental Disorders in India (weighted percentage)

The above chart shows the percentage of various mental health disorders in the Indian population. Even though depression accounts only for 2.7% the hidden burden of this disease is larger as SUDs and anxiety spectrum of diseases eventually lead to depression. Also it is a notable finding that suicidal risk in Indian population is 0.9% which is also an indirect measure of depression.

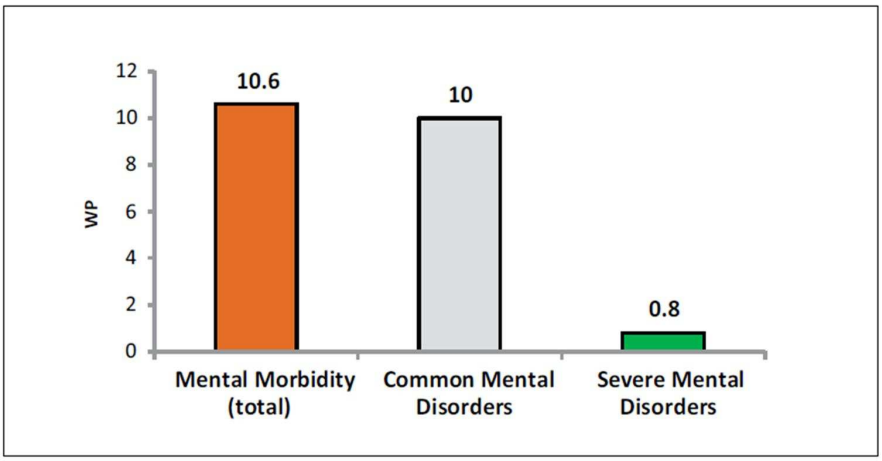


Figure 15. Current Prevalence of Common and Severe Mental Disorders

Thus the weighted prevalence of MDD is 5.2% for lifetime and 2.7% currently. This accounts for 1 in 20 people are suffering from depression currently and 1 in 40 people have a past history of depression.

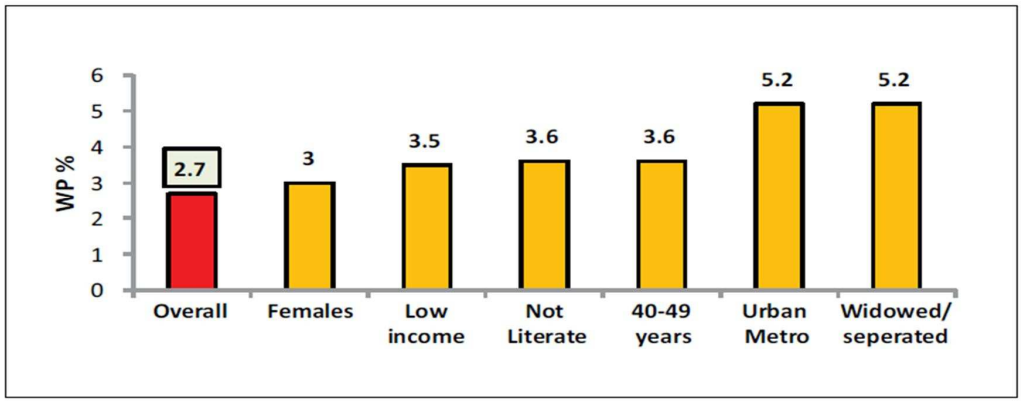


Figure 16. Prevalence of MDD

Suicide, Depression and cytokines :

Depression and Suicide – Biological perspective:

MDD is regarded as a biologically inherited disorder running in families. The evidence for this is got from Family, Twin and adoption studies that gave converging results. There was 30-40% association of genetic factors with the gene- environment interaction explaining the rest.[43]

Based on this many biological theories have been proposed into which different subsets of patients fit. One such theory is the Monoamine deficiency theory that focuses on the depletion or imbalance of monoamines particularly serotonin in mood regulating areas like hippocampus, prefrontal cortex etc.[44]

The neurotrophic hypothesis states that there is considerable loss of hippocampal volume with every episode of depression. Preclinical studies have given some evidence towards this hypothesis with low levels of BDNF expression in hypothalamus associated with depressive behavior.[45,46]

The stress hormone hypothesis talks about the role of HPA axis in depression. Hyper reactivity to psychological stress may be a result of childhood trauma. This is evidenced by increased CRH levels in victims of childhood trauma who present with depression.[47] CRH levels are also increased in the CSF of depressed patients.[48] Also there is an increase in corticotrophin neurons in the limbic areas as evidenced by the postmortem studies which may be a response to the increased levels in the periphery.[49]

A convergent evidence to the role of CRH is obtained by establishing the role of CRH in producing vegetative symptoms like lack of appetite,distrupted sleep, altered psychomotor functioning etc. that resemble depression.[50]

The same explanation holds good for the role of cytokines. Sickness behavior in one condition which shows symptoms of high resemblance to depression. Fatigue, anhedonia, irritability, psychomotor retardation, cognitive impairment are common to both sickness behavior and depression. As this behavior results due to elevations in pro inflammatory cytokines IL1β, TNFα and IL 6 there is a possible role of these cytokines in depression also.[51]

Exploring the biological aspects of suicide is very difficult. Most of the evidences are got from psychological autopsy studies and postmortem studies. Furczyk et al[52] did a literature review of postmortem studies of suicide. The findings could be summarized as follows.

Studies on HPA axis shows increased levels of CRH and POMC mRNA levels with decrease in their receptors. Studies on neuroplasticity shows decrease in BDNF and NT-3 levels along with their receptors. Neurotransmitter studies showed upregulation of Serotonergic, Gabaergic and Endocannabinoid receptors along with downregulation of Glutamatergic, Dopaminergic and Noradrenergic receptors. Cell signaling pathway studies shows increase in IL 1β, IL 3,IL 4, IL 6, IL 13 and TNFα.

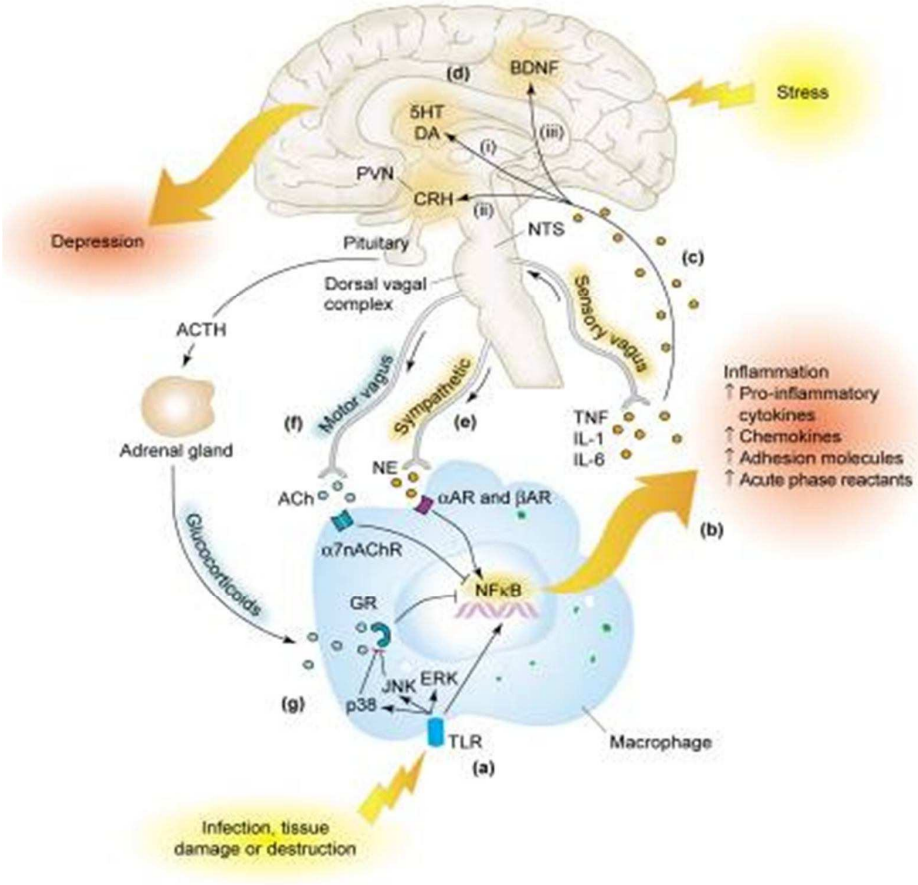


Figure 17. Neurocircuitary of Depression

Cytokines-mechanism of action in depression

Cytokines are found to be involved in almost every aspect of pathophysiology of depression-the neurotransmitters and neuroendocrine metabolism and also in neural plasticity. Moreover inflammatory cytokines are potent activators of Nuclear Factor kappa B(NFκβ), a primary transcription factor involved in inflammation ,which leads to altered Glutamate metabolism and oxidative damage as a consequence of its excitotoxicity with loss of glial elements.[53]

Cytokines being large polypeptides of approximate molecular weight of 15-25 kD, there arises a question of how the peripheral circulating cytokines exert action on CNS.

Quan N and Bank WA[54] has formulated an acceptable answer to this query. Through animal experiments they have postulated four possible mechanisms for entry of cytokines to brain cells.

1) passing through leaky channels of BBB
2) actively transported through transport molecules
3) producing cytokines locally through activation of perivascular macrophages and lining endothelial cells of blood vessels
4) relay of signals through binding with receptors in the peripheral nerves like vagus that form the afferent pathway to certain regions like NST and hypothalamus involved in behavioural mechanisms.

The hippocampus that is concerned with learning , processing of learned material and consolidating the learned material into memory is one area of brain which shows continuous neurogenesis even in adult life so as to facilitate the neuroplasticity necessary for the above said purposes. Establishment of new neuronal circuits in hippocampus, hypothalamus and the prefrontal cortex is necessary for learning through new emotional situations learned in day to day life and this determines the reactivity of the individual to such emotional situations in future.

So the concept of neuroplasticity plays an important role in genesis of depressive symptoms. When there is aberrant circuit formation due to loss of neuronal function or defective neuroplasticity, the emotional reactivity of the individual get altered and results in psychotic withdrawal, depression , mania,phobia etc.[55]

Brain Derived Neurotrophic Factor (BDNF) is a well known neurotrophin that plays a very vital role in neuroplasticity in brain during adult life. The expression of BDNF is influenced by the cytokines. Most common cytokines involved are inflammatory cytokinesareIL-1β, IL-6,TNF-α and IFN-γ.[56]

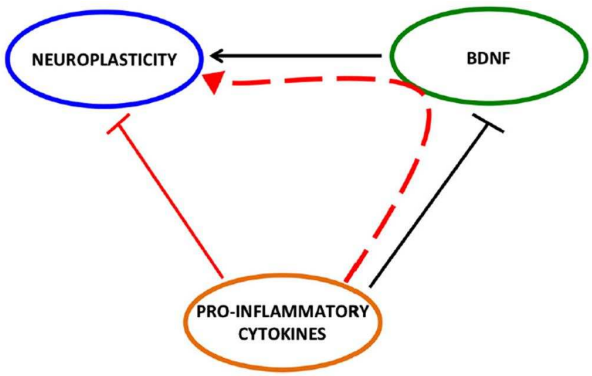


Figure 18. Neuroplasticity,BDNF and Cytokines

Among these cytokines IL 6 is implicated in almost all neuroprotective pathways related to neural plasticity, neurogenesis, Long TermPotentiation, and memory.[57]

Monje et al[58] did animal studies regarding the relation between cytokines and neurogenesis. They found that there was profound inhibition of neuronal

differentiation and decrease in neuronal survival on injection of LPS intraperitoneally in rats. LPS is a potent inducer of IL 6 production.

The same authors also incubated hippocampal progenitor cells with recombinant IL 6 and demonstrated that there was a decrease in neurogenesis by 50% and lack of differentiation of neuronal cells.

These effects of IL 6 on neuronal plasticity are probably mediated through altered expression of BDNF transcription factors and thus gene expression.[59] For example there was a decrease in levels of BDNF mRNA in the hippocampus of rats after 4 hours of intraperitoneal injection of IL 1β or LPS that are potent inducers of IL 6.[60]

These negative effects of cytokines on BDNF have notable implications in many a variety of pathological conditions in which the hippocampus dependent memory plays a crucial role like dementia , depression etc.[61] It is also a well known fact that BDNF plays a etiological role and is also a treatment strategy for many neuropsychiatric conditions like depression.[62,63]

In short the relation among depression, inflammation and BDNF could be summarized as in the following figure.

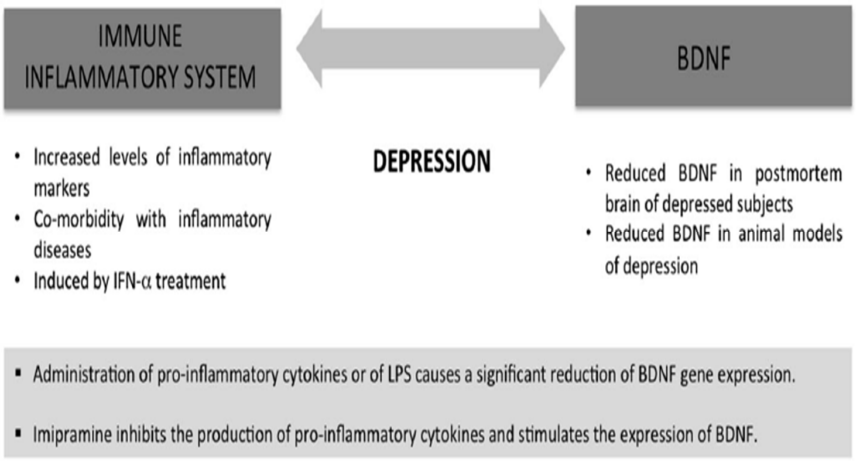


Figure 19. Cytokines ,BDNF and Depression

Stress induces changes in HPA axis which stimulates the immune system and increase the production of pro inflammatory cytokines such as IL 1β, IL 6 and TNF α.[64]

Smith RS[65] in his macrophage theory of depression states that excessive secretion of macrophage monokines leads to hormonal abnormalities that are supposed to cause depressive symptoms. He explains the presence of depression as a comorbid finding in chronic inflammatory diseases like type 2 diabetes , rheumatoid arthritis, myocardial infarction etc. through this theory. He also proposed that the higher incidence of depression in females may be due to stimulant action of estrogen on macrophages.

Michael R Kraus et al[66] studied the psychiatric side effects of Interferon α treatment to patients with Hepatitis C. There was an increase in depressive symptoms in those patients receiving the drug. Higher correlations were found with anger and hostility scores. This is consistent with the hypothesis of altered immune system in the genesis of depression and suicidal behavior

Coelho et al[67] have studied the effects of Glucocorticoids as treatment for endotoxin induced fever in rats and have found that the acute phase reactants IL 1 and IL 6 that cause fever directly act upon the Glucocorticoid receptors in the Hypothalamus and stimulate the HPA axis that results in increased production of Glucocorticoids mainly cortisol. This cortisol apart from reducing fever when present in excess quantities can cause depressive symptoms.

Maes M[68] in his review paper have concluded that patients with major depression show activation of immune system particularly the inflammatory mechanism involving the phagocytes, activated T cells, proliferation of B cells , autoantibody production, prostaglandin synthesis and in particular increased positive acute phase proteins like IL 1 and IL 6. He has also hypothesized that these acute phase proteins are responsible for the HPA axis hyperactivation, dysregulation of serotonin metabolism and vegetative symptoms of depression.

The same author with a group of others has published another paper subsequently stating that plasma concentrations of IL 6 were raised in depression and it also correlated with sIL 6 receptors. The antidepressant treatment does not

reduce the cytokine levels and this may explain resistance to antidepressants in a subgroup of patients.[69]

Musselman DL et al[70] studied the IL6 levels in cancer patients. They grouped the study subjects into four groups- physically and mentally healthy controls, physically healthy depressed patients, cancer patients without depression and cancer patients with depression. The results showed an elevated plasma IL 6 levels in cancer patients with depression comparable to healthy depressed patients.

In a meta analysis was done by Dowlati Y et al [71] among 24 studies that analysed various cytokine levels in depressed patients. Out of those 24 studies 16 of them showed results for elevated IL 6 in depression while only fewer studies concluded positive results for other cytokines. The other cytokines were TNFα. IL 2,IL 1β,IL 4,IL 8,IL 10 and IFNγ. Thus Il 6 is the frequently associated cytokine in depression.

Bob P et al[72] studied the correlation of IL 6 levels and BDI scores along with traumatic stress symptoms and dissociation symptoms. The serum IL 6 levels significantly correlated with BDI scores and stress scores.

Dahl J et al[73] have done a crossover study with 50 drug naïve depressed patients and 34 healthy controls. They measured the plasma levels of nine cytokine - interleukin (IL)-1β, IL-1 receptor antagonist (IL-1Ra), IL-5, IL-6, IL-7, IL-8, IL-10, granulocyte colony-stimulating factor (G-CSF), and interferon

gamma (IFNγ) at the baseline which were found to be increased in comparison with the healthy controls. After 12 weeks of antidepressant treatment the levels of seven of these cytokines-IL-1Ra, IL-6, IL-7, IL-8, IL-10, G-CSF, and IFNγ significantly decreased.

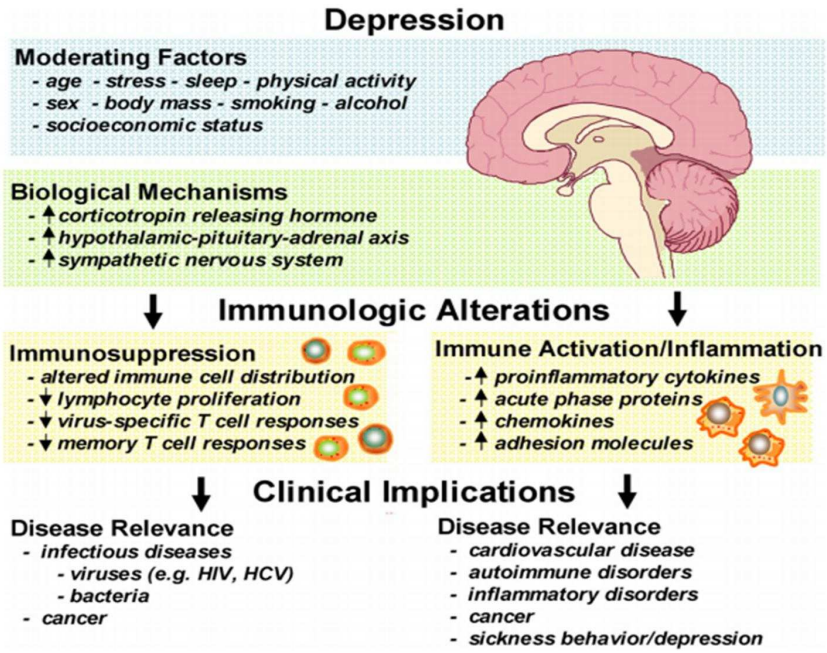


Figure 20 : Immunological Alterations in Depression

Raison et al[74] in their paper on inflammation and pathogenesis of depression has stated that depression and cytokines are linked through the stress-immunity pathway. According to them both physical and psychological stress activate the signaling pathways of the proinflammatory cytokines in the areas of brain concerned with emotional regulation. But in contrast stress also causes suppression of acquired immune responses as seen in physical illness and depression so ithe role of immune mechanisms in depression can be summarized in the following figure.

Dantzer et al[75] talks about the possible mechanism by which cytokines influence the development of depressive symptoms both in animal models and humans. The activation of immune system either by immunotherapy or by endogenous processes alter the tryptophan metabolism. Tryptophan is the precursor of serotonin. The metabolism of tryptophan is as follows.

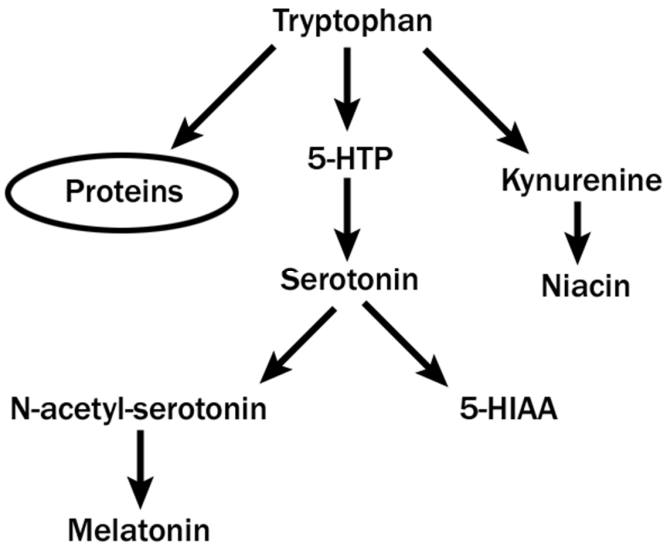


Figure 21. Pathways of Tryptophan metabolism

The enzymes responsible for the kynurenine pathway are tryptophan 2,3 dioxygenase (TDO) and indoleamine 2,3 dioxygenase (IDO). The proinflammatory cytokines induce these enzymes and activate the kynurenine patway so that tryptophan is not available in sufficient quantities for production of serotonin which is the principle neurotransmitter involved in depression

Renee D.Goodwin and William W.Eaton[76] in a epidemiologic catchment area study found out that there is increased suicidal ideation and lifetime suicidal

risk in asthmatic patients. As asthma is a disease of hypersensitivity of the immune system this finding directs to the possibility of immune dysregulation in suicidal patients.

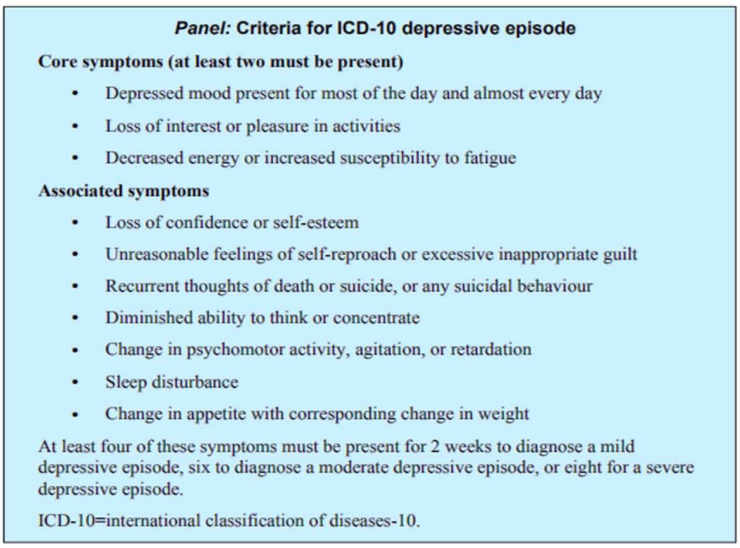
Janelidze S et al[77] have done a study among 47 suicidal patients in comparison with 17 non suicidal depressed patients and 16 healthy controls. They measured plasma IL2,IL 6 and TNFα in all the subgroups. There was increase in IL 6 and TNFα levels and decrease in IL 2 levels in suicidal patients when compared to the other groups.

Thapar A et al[78] published a paper on depression on adolescence. According to them depression is the foremost reason for suicide in adolescents and almost half of the suicide victims were found to have suffered from depression at the time of death.individuals younger than 25 yrs being treated for depression are more likely to attempt suicide.

Mackenzie S et al[79] screened around 1622 college students for depression and suicidal ideation using BDI PC which provides a subscale for suicidal ideation. When the results were analysed 26.4% of the females and 24.7% of the males scored positive for depression. Overall 10% of females and 13% of males expressed suicidal ideation. Only 2.2% of females and 2.8 % of males who scored negative on BDI PC expressed thoughts of suicide while 31.7% of females and 39.9% of males with high depression scores also had suicidal ideation.

Pompili et al[80] studied 74 inpatients admitted with MAD in psychiatric wards for suicide intent. They concluded that 52% of the patients who scored high on hopelessness and impulsivity scores had high suicidal intent. 36.5% of those who score high in depression scale reported previous suicide attempt and 21.6% reported multiple attempts.

Also the ICD 10 diagnostic manual[81] lists suicidal behavior and even thoughts of death or suicide as one of the diagnostic criteria for depressive episode.



Panel: **Criteria for ICD-10 depressive episode**

Core symptoms (at least two must be present)

- Depressed mood present for most of the day and almost every day
- Loss of interest or pleasure in activities
- Decreased energy or increased susceptibility to fatigue

Associated symptoms

- Loss of confidence or self-esteem
- Unreasonable feelings of self-reproach or excessive inappropriate guilt
- Recurrent thoughts of death or suicide, or any suicidal behaviour
- Diminished ability to think or concentrate
- Change in psychomotor activity, agitation, or retardation
- Sleep disturbance
- Change in appetite with corresponding change in weight

At least four of these symptoms must be present for 2 weeks to diagnose a mild depressive episode, six to diagnose a moderate depressive episode, or eight for a severe depressive episode.

ICD-10=international classification of diseases-10.

Figure 22. ICD-10 Criteria for Depressive Episode

So from the above discussion it is obvious that cytokines have a definite role in pathophysiology of depression and as it is the leading cause of suicide implies that cytokines particularly IL 6 is involved in suicidal ideation and attempts.

Interleukin 6 as biomarker of depression:

So now the question arises whether IL 6 could be considered as the peripheral biomarker of depression. HD Schmidt et al[82] have done a thorough research on the biomarker panel of depression. The following picture gives a view of different classes of biomarkers of depression and the basic principle of their detection in blood.

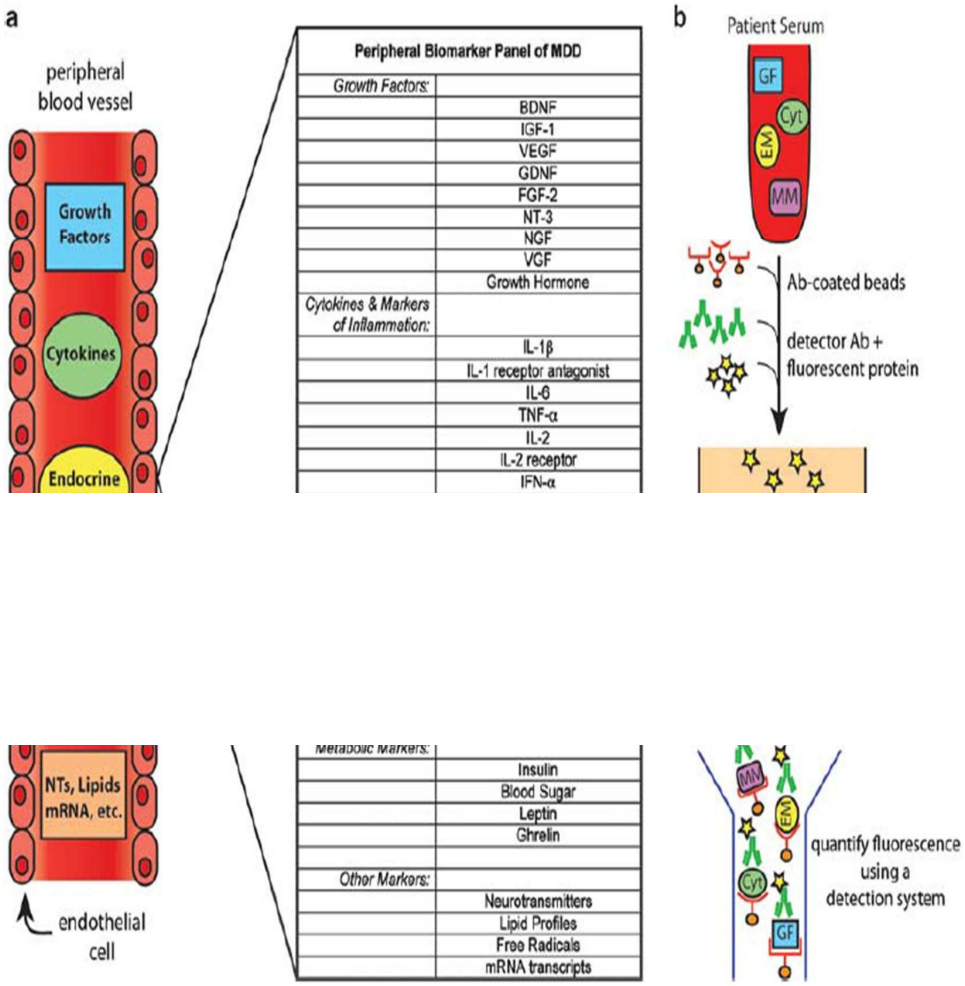


Figure 23. Biomarker Panel of Depression.

The authors have stated that among the cytokine markers the pro inflammatory cytokined IL 6 and TNFα are the key elements in depression. These cytokines act on the hippocampus to inhibit neurogenesis which inhibits the responsiveness of hippocampus to antidepressants. The elevated peripheral blood levels of IL 6 comes down after treatment with antidepressants. Also TRD patients have increased levels of IL 6. Thus serum Il 6 levels could be used to detect depression and to track the treatment response means that it could be used for both diagnostic and prognostic purposes.

Suicide, depression and lipids:

S. Cham et al[83] published a case series on neuropsychiatric side effects of statins - the lipid lowering drugs. The problems that were reported by the participants are violent ideas, irritability, depression and suicidal thoughts that resolved on discontinuation of the drug to return back on reinitiation of treatment. The causality criteria used was Naranjo criteria. According to this criteria 4 out of the 12 participants fitted into the definite causality category; 5 fall into probable category and the remaining in the possible category.

Kim YK and Myint AM[84] explored relation between serum cholesterol and suicidal risk in depressed patients. They measured serum cholesterol in 149 MDD patients admitted for attempting suicide and 149 MDD patients with no history of suicide attempts and compared them with 251 normal controls after matching them for age,gender,BMI and total serum protein. The serum TC levels

were significantly lowered in suicidal patients than the non suicidal depressed patients. Thus they conclude that low cholesterol can be used as an indicator of suicidality in MDD patients.

Ellison LF and Morrison HI[85] did a retrospective analysis of mode of death in 11,554 participants of nutrition Canada survey. Among these 27 reported death due to suicide. When stratified analysis of this data was done six times higher suicidal risk was present in the lowest quartile group of serum cholesterol.

Messaoud et al[86] investigated the suicidal risk in suicidal MDD patients. They measured lipid profile in 162 MDD patients out of which 52 had history of suicide attempts. Controls were 151 healthy subjects. There was a decrease in total cholesterol in the suicidal group while other parameters TGL, LDL, HDL were normal.

Cytokines and Lipid metabolism:

Cytokines particularly the proinflammatory category are acute phase reactants. The IL 6 which is the principle mediator of acute phase reaction exerts its action by inducing and inhibiting the hepatocytes as depicted in the picture below.

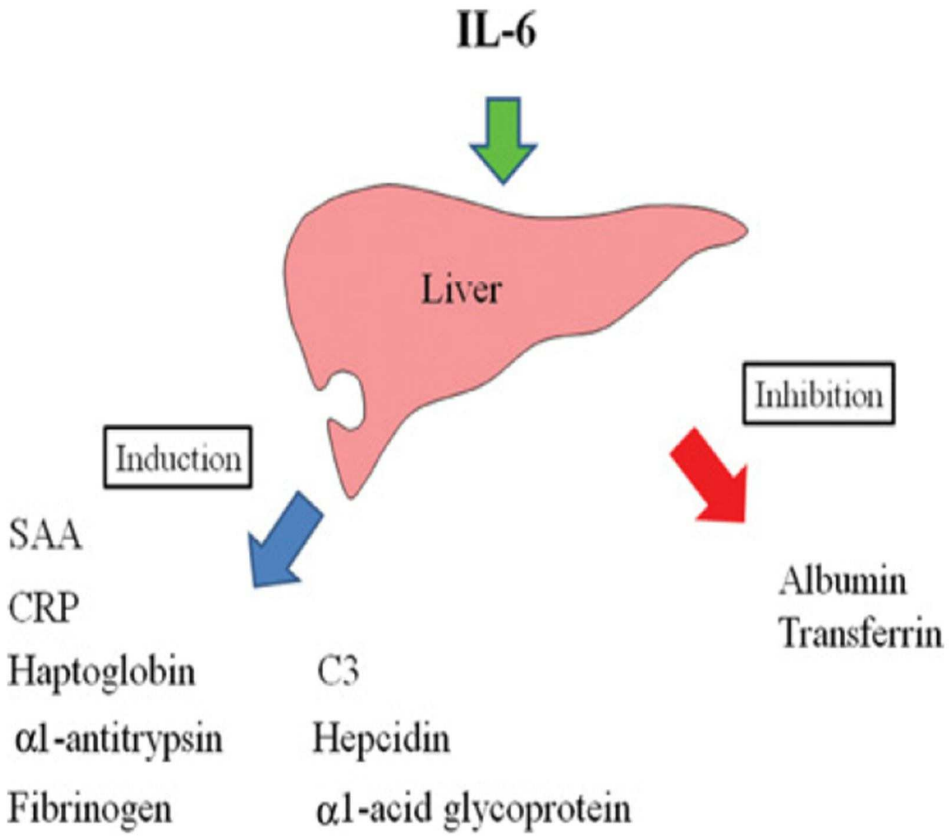


Figure 24. Action of IL 6 on Liver

Similarly IL 6 also has a significant role in lipid metabolism through increase in uptake of lipoproteins into the tissues and inhibiting hepatic lipogenesis. Animal studies showed a decrease in lipid levels in IL 6 transgenic mice when compared to their littermates.[87]

Treatment with IL 6 receptor antibodies restored the lipid levels. Also the chronic inflammatory diseases like Rheumatoid arthritis in their acute phase show a hypolipidemic profile which gets corrected with ongoing treatment.[88]

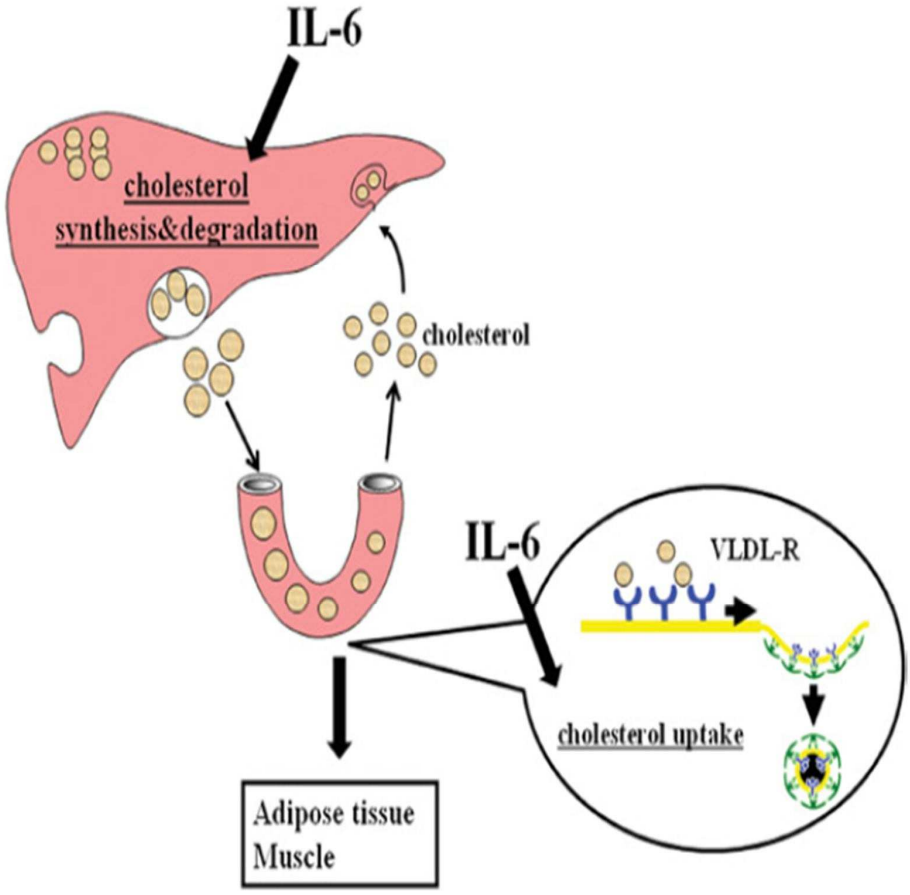


Figure 25. Action of IL 6 on Lipid metabolism

When exploring the reasons for this hypolipidemia, upregulation of VLDL receptors is consistently associated with these conditions that shows increase in IL 6 levels.[89] as illustrated in the figure 25.

Suicide, Depression and autonomic functions:

Autonomic nervous system[90]

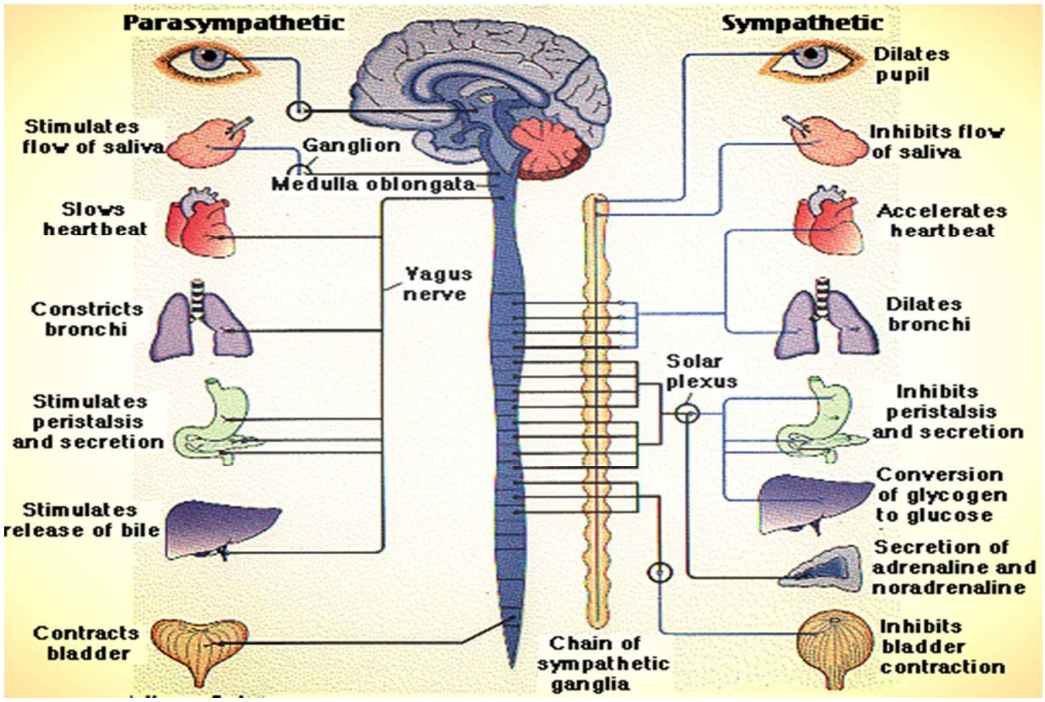


Figure 26. Autonomic Nervous System

The ANS consists of sympathetic and parasympathetic nervous system. It innervates almost all organs of the body and is responsible for maintenance of homeostasis. The innervations is divisible into preganglionic and postganglionic neurons. The chain of ganglia are present along the spinal cord.

Depending upon the location of the preganglionic neurons the parasympathetic syatem is called craniosacral outflow and sympathetic system is called thoracolumbar outflow. Acetylcholine is the principle neurotransmitter of parasympathetic pathway while in addition to Ach the catecholamines are also released in the sympathetic pathway.

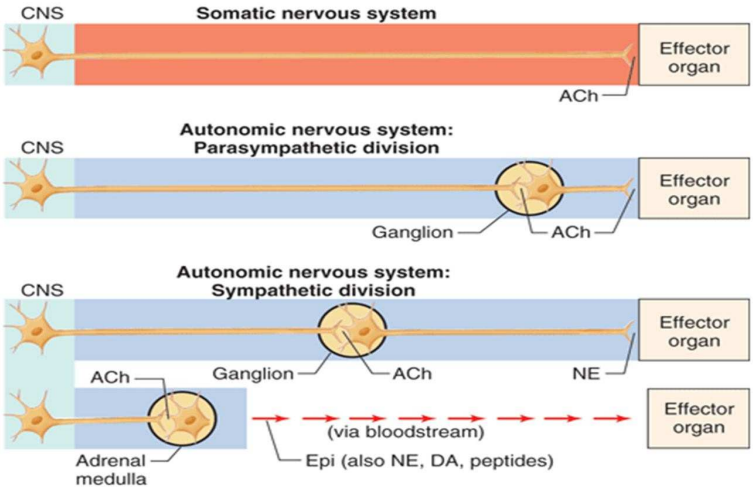


Figure 27. ANS in comparison with Somatic nervous system

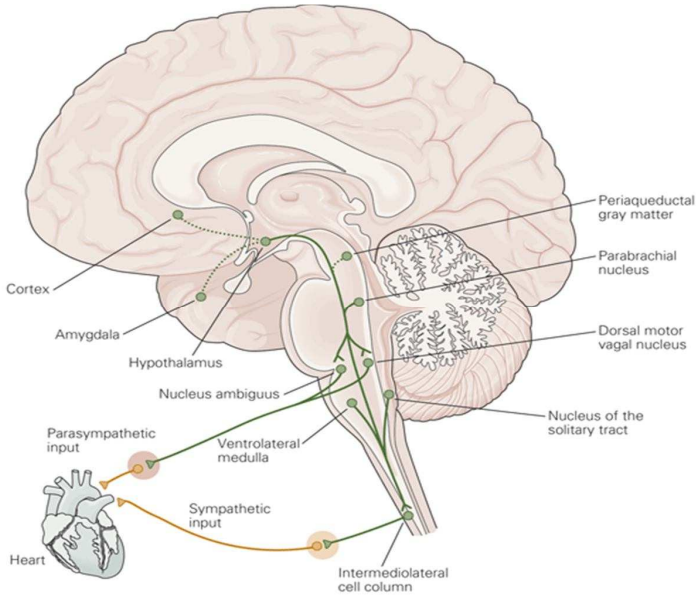


Figure 28. Action of ANS on Heart

The figure 28 shows how cardiac autonomic functioning is controlled by the CNS. The pathways involve the areas concerned with emotional regulation like hypothalamus, amygdale and nucleus ambiguous. Thus the emotional dysregulations affect cardiac activity and cardiac pathology indicate emotional disturbances.

With respect to the heart the sympathetic nervous system through the stellar ganglion innervates the cardioaccelerating centre and the parasympathetic system innervates the cardiomoderator centre through the vagus.

Both these divisions of ANS operate in an antagonistic manner to dynamically modulate the vital functions. The interactive modulation of the functioning of the SA node maintains the normal beat-to-beat variability in heart. Vagal activity is the primary modulator of heart rate.

This sympathovagal balance can be measured by HRV. It is an indicator of CNS control over ANS and the feedback integrity of peripheral neurons to the central level.

ANS shows high levels of adaptability to physical and psychological stress. Thus pathologies induced by stress indicate a less flexible rigid autonomic nervous system.[91]

Depression has been related to increased cardiovascular morbidity and mortality.[92]

Following a diagnosis of coronary artery disease depressed patients are more likely to report a major cardiac event like angina, acute MI etc.[93]

Though the relationship between depression and cardiac events is well established the cause for this relationship is uncertain. Dysregulation of

autonomic nervous system evidenced by increasing sympathetic activity and decreased parasympathetic activity is one of the most acceptable explanations[94].

Roy et al[95] studied the levels of NE and its metabolites in plasma, CSF and urine on 140 depressed patients and 140 healthy controls in relation to plasma cortisol after dexamethasone challenge. There was an increase in the catecholamine levels and their metabolites. So they concluded that apart from the HPA axis abnormalities depression also shows high levels of this neurotransmitter and its metabolites.

As NE is the neurotransmitter of sympathetic nervous system, increased levels may be interpreted as sympathetic overactivity. But the antecubital venous sampling done in this study may reflect only the local sympathetic activity.

Veith et al.[96] demonstrated an increase in plasma NE, in MDD patients who were otherwise healthy ,in arterialized venous samples using plasma NE kinetic techniques based on the principle of radioactivity assay which is a more reliable measure of postganglionic NE release and clearance .

The frequently used physiological investigation to measure the cardiac autonomic function is heart rate variability analysis. As the ANS modulates the beat-to beat variability in the cardiac rhythm it is regarded as the reflection of sympatho-parasympathetic balance that regulates the heartbeat. Thus low HRV is interpreted as excessive sympathetic drive of the heart along with inadequate parasympathetic modulation or both. [22]

Rechlin T et al[97] made a standardized HRV aanlysis in four group of subjects-MDD patients, panic disorder, reactive depression with suicidality and normal subjects. No difference was seen between reactive depression and normals. Panic disorder shows sympathetic overactivity evidenced by higher heart rate and increased LF. MDD group showed significant decrease in parasympathetic activity with low HRV and HF in power spectrum analysis.

Koschke M[98] et al did HRV analysi on MDD patients and concluded that these patients had decreased baroreflex sensitivity and parasympathetic activity while there was an increase in QT variability influenced by sympathetic nervous system.

Udapa K et al[99] measured HRV in MDD patients and found that there was decreased valsalva ratio, maximum/minimum ratio and greater sympathovagal imbalance in comparison with healthy controls.

Thus, based on the above evidences we are convinced that there is a causual relationship between Inflammation, Lipid metabolism and Autonomic dysregulation at one end and depression with suicidality at the other end.

With this background we proposed to do a study on patients who attempted suicide for the first time to find out whether increase in IL 6 levels, decrease in cholesterol levels in the serum along with autonomic imbalance are related to depression scores on BDI II.

Aim and Objectives

AIM AND OBJECTIVES

AIM :

- To determine whether autonomic dysfunctions and Interleukin- 6 mediated changes in Serum Lipid Profile can be used as Bio-Marker for predicting depression in first attempt suicide patients.

Objectives :

1. To measure the levels of serum interleukin-6 in first attempt suicide patients.
2. To measure the serum lipid profile
3. To correlate the serum Interleukin-6 and Serum Lipid Profile levels
4. To assess the depression in first attempt suicide patients using BECK'S DEPRESSION INVENTORY II
5. To assess the intent of committing suicide using BECK'S SUICIDE INTENT SCALE
6. To correlate the Serum Interleukin-6 and Serum Lipid Profile levels with severity of depression and suicidal intent.
7. To assess the autonomic dysfunctions using Ewing's Battery of tests.

Materials and Methods

MATERIALS AND METHODS

This is an observational cross-sectional study conducted during the year 2016-17 in Rajiv Gandhi Government General Hospital attached to Madras Medical College.

Subjects for this study were recruited from the Institute of Internal Medicine, RGGGH.

Study was conducted in the human experiments laboratory attached to the Institute of Physiology and Experimental medicine.

Subject selection:

Patients of both genders, in the age group of 20-40 yrs, admitted to the RGGGH with history of first attempt of suicide in any modality were included in the study.

Patients with:

- Past and present history of any major or minor psychiatric illness.
- Previous history of attempted suicide.
- Chronic inflammatory diseases.
- Diabetes and malignancy.
- Recent history of fever, injury and infections. Patients on statins
- History of any condition that is found to cause autonomic disturbances (ex. cardiovascular events) were excluded from the study.

After scrutinizing the above inclusion and exclusion criteria 51 subjects of both genders were recruited into the study.

An informed consent in both verbal and written form was got from the participants after explaining the objectives and procedure in detail.

After this the consenting participants were subjected to the following set of investigations.

1. Serum interleukin levels
2. Serum lipid profile.
3. Ewing's battery of autonomic function tests.

Sample collection:

Under universal sterile precautions 5 ml of venous blood sample was collected in the overnight fasting state for estimation of serum IL 6 and Lipid Profile. This sample is centrifuged within 30 minutes of collection at 3000 rpm for 10 secs. To separate the serum. These serum samples were stored at -20°C in a well maintained deep freezer.

When adequate samples were collected they were sent to the laboratory for analysis.

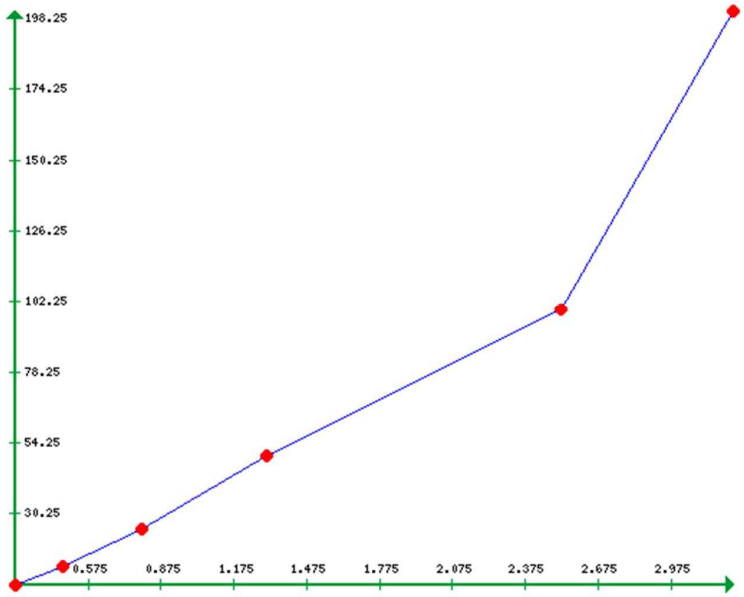
ESTIMATION OF SERUM IL 6

Serum samples stored in separate eppendrof tubes were analysed in the Department of Immunology, Tamilnadu Dr, MGR medical university.

Method used was solid phase sandwich ELISA with a Diaclone kit used for research purposes. The kit consists of a 96 well microtiter plates coated with capture antibody highly specific for IL6. Standard and control are provided in the kit. Standards are prepared using the given diluents in seven different dilutions. Five wells were used for control. One well is zero standard. Remaining wells were used for the samples. In total 64 wells were used for analysis and remaining wells were used to duplicate extremes of values. After adding the samples , standard and control a biotinylated anti-IL-6 secondary antibody is added to all the wells and incubated at room temperature. After washing the excess solution with the buffer provided a HRP conjugate is added and incubated. Washing is again repeated and a chromogen substrate is added which results in the development of blue colour. The colour development was stopped using an acid that changes the colour of the final product to yellow. The absorbance of the coloured complex is measured in a spectrophotometer with 450 nm as primary wavelength and 620 nm as reference wavelength.

A linear standard curve was generated by plotting the average absorbance of each standard on the verti cal axis versus the corresponding IL-6 standard concentration on the horizontal axis.

Using this standard curve the OD values of each sample is extrapolated against the IL 6 standard concentrations to determine the amount of IL 6 in each sample.



Graph 1. Standard curve of IL 6 analysis

The unit of measurement is pg/ml and the sensitivity of the kit was 2 pg/ml.

ESTIMATION OF SERUM LIPID PROFILE

Fasting serum lipid profile was done at the clinical central laboratory at RGGGH attached to the Institute of Biochemistry, Madras Medical College. The kit used was tics Cobas CHOL Gen.2, HDLC Gen.3 and TRIGL kits from Roche Diagnostics. The method was automated Electrochemiluminescence. The serum total cholesterol, HDL and triglycerides were estimated and the serum LDL, TC/HDL ratio and LDL/HDL ratio were calculated using those values.

Principle of the test:

This is a calorimetric method that depends upon enzymatic reaction. The substrate undergoes oxidative reaction to produce intermediate products. These electrochemically produced intermediates are acted upon by peroxidase enzymes which results in the formation of a coloured dye. The colour intensity of the dye is directly proportional to the concentration of cholesterol in the sample. The reagents for the different types of cholesterol are different and are provided in the kit .

Assay procedure:

Prior to loading the samples to the automated Cobas analyser calibration was done. Measurement of controls done after calibration. When the samples were loaded the desired test is chosen in the computer. The analyser completes the test and data can be retrieved from the host computer.

Normal levels according to this assay are

- Total Cholesterol < 200 mg/dl
- HDL 35.3-79.5 mg/dl
- LDL <100 mg/dl
- TGL 40-160 mg/dl

ANALYSIS OF HRV

Heart rate variability of the selected subjects was recorded and analysed using the Physiopac 8 channel HRV recorder. This instrument is widely used for research purpose and has an in built software for analysis of the recorded data.

Procedure

Instructions to the subject:

- After explaining to the subject about the procedure and purpose of recording the HRV in understandable language consent was obtained.
- A convenient date was fixed up for the procedure.
- The following set of instructions were given to the participants
- To get a good sleep the day before the recording
- To take breakfast 2 hrs prior to the test.
- To avoid Caffiene, Nicotine and Alcohol 2 hrs before the test.
- To be dressed up in comfortable loose clothing .
- To remove any accessories and jewelery that may interfere with the recording
- To report with an attender.
- To empty bladder just before the test.

Recording was ideally done in the morning hours between 10 am-12 noon. The room was made as quiet as possible and the temperature was maintained at an optimum level of 25-28°C in a subdued lighting. All mobile phones were switched off.

Prior to the test all the maneuvers involved were taught to the participant by demonstration and they were allowed to practice them till they get the precision. All the vital parameters like pulse rate, blood pressure, temperature and respiratory rate were noted down.

Then the participant was made to lie quietly in the supine position with eyes open and to relax for about 10- 15 mins before connecting the electrodes.

Electrode placement:

Limb leads were used in this test. The area where the leads are to be placed was cleaned thoroughly with spirit and the specialized leads were attached to all the four extremities. They are the connected to the ECG recorder attached to the Medicaid 8 channel Physiopac. The lead II is chosen for recording. ECG is recorded at this resting state for 10 mins. Then it is saved for analysis.

Then for measuring the autonomic functions the participant is subjected to the Ewing's Battery of tests which is considered as gold standard to measure the autonomic dysfunction.[100]

The tests included in this battery are

1. Heart rate response to the Valsalva manoeuvre (VR ratio)
2. Heart rate response to standing up (30:15 ratio or Posture ratio)
3. Heart rate response to deep breathing (E/I ratio)
4. Blood pressure response to standing up (OST-Orthostatic Standing Test)
5. Blood pressure response to sustained handgrip (IGHT- Isometric Hand Grip Test)

The Heart rate response tests are the measure of parasympathetic cardio vascular regulation. The Blood pressure responses are the measure of sympathetic adrenergic vascular regulation. These tests are done while the HRV is continuously recorded with an interval of 2 minutes of resting measurement in between subsequent maneuvers.

Heart rate response to deep breathing (E/I ratio):

It is called Expiration Inspiration ratio or deep breathing test. The subject is asked to lie down in the supine position comfortably and take deep breaths of inspiration lasting for 5 secs. and expiration lasting for another 5 secs. This would complete 6 cycles of deep breathing in one minute. The mean of the longest R-R interval during expiration and the mean of the shortest R-R interval during inspiration is calculated. E/I ratio is calculated using the formula

$$\text{E/I ratio} = \frac{\text{Mean of longest R-R interval during expiration}}{\text{Mean of shortest R-R interval during inspiration}}$$

Heart rate response to standing up (30:15 ratio or Posture ratio):

The HRV is recorded first in the supine relaxed position. After 2 mins of recording the subject is asked to stand up when the recording is continuously going on. The longest R-R interval occurring in between the 20^{th} – 40^{th} seconds after standing is noted and the shortest R-R interval occurring between the 5^{th} – 25^{th} seconds after standing is noted . the posture ratio is calculated with the following formula.

$$30:15 \text{ ratio} = \frac{\text{longest R-R interval during 20-40 seconds after standing}}{\text{shortest R-R interval during 5-25 seconds after standing}}$$

Heart rate response to the Valsalva manoeuvre (VR ratio):

The subject is asked to forcefully expire into a mercury manometer to sustain a pressure of 40 mmHg for 15 seconds. The ratio of the longest to shortest R-R interval occurring during this manoeuvre is calculated.

Blood pressure response to standing up (OST-Orthostatic Standing Test):

The blood pressure was measured when the subject was lying down in supine position and fully relaxed. Then he/she was asked to suddenly stand up and lean against the wall. The blood pressure was recorded in the first and third minutes of standing up. The systolic BP which is reduced in the first minute of standing is taken for analysis. The difference in BP is calculated by the following formula.

$$\text{OST } SBP_1 = \text{SBP in supine position} - \text{SBP in the first minute after standing}$$

Blood pressure response to sustained handgrip (IGHT- Isometric Hand Grip Test):

As in the OST blood pressure is first measured in the supine relaxed position. Then the subject was asked to perform a simple exercise. A hand grip dynamometer was used for this purpose. First the was told to press the

dynamometer with the maximum possible force. This was done three times and the highest reading was taken as the maximum voluntary contraction (MVC). Then the subject was instructed to maintain 30% of MVC as long as possible within a period of 5 minutes. The blood pressure was recorded in the opposite arm during and after the manoveure. An increase in Diastolic BP was recorded and calculated as follows.

IGHT DBP_1 = DBP after first minute of the manoveure – DBP at rest

Thus the autonomic functions were measured using the Ewing's Battery of tests for dysautonomia.

Results were scored as normal and abnormal as follows.[101]

Table 2. Normal and Abnormal values for Ewing's test

TEST	NORMAL	ABNORMAL
E/I ratio	Above age related reference values	Below age related reference values
30:15 ratio	≥ 1.04	≤ 1.00
VR ratio	≥ 1.21	≤ 1.10
OST SBP_1	Decrease of ≤ 10 mmHg	Increase of ≥ 30 mmHg
IGHT DBP_1	Increase of ≥ 16 mmHg	Decrease of ≤ 10 mmHg

SCORING DEPRESSION

The study participants were screened for depression using the Beck's Depression Inventory II which is most widely used instrument for research purpose world wide. It is a 21 item scale with four options under each item ranging from 0-4. Thus the range of total score is 0-64. It is a self rated scale which takes about 10-15 minutes to complete. It covers almost all domains in the symptom complex of depression. So it is very widely used for screening depression in both clinical set up and research purpose. The original version was introduced by Beck et al in 1961, validated both in general practice and clinical trials. Periodically the scale is being revised according to the changes made in the DSM criteria for diagnosis. The interpretation of the scores were done in the following manner.[102,103]

Table 3. Scoring of BDI II

Total score	Levels of depression
0-10	Normal
11-16	Mild mood disturbance
17-20	Borderline clinical depression
21-30	Moderate depression
31-40	Severe depression
Above 40	Extreme depression

SCORING OF SUICIDE INTENT

The subjects were also rated for the intention to die using a questionnaire Beck's Suicide Intent Scale. This is a 20 item questionnaire out of which last 5 items are not used for scoring. First 8 items are scored by the observer according to the nature of act and the history. Items 9-15 are subjective measures and self reported by the patient. Each item is rated between 1-3. Thus the range of total score is 15-45. There is no normal rating as attempting suicide itself is considered as abnormal. Scoring is done as follows.[104]

Table 4. Scoring of SIS

Total score	Suicide Intent
15-19	Low Intent
20-28	Medium Intent
29+	High Intent

As mentioned above each one of the participant was subjected to a detailed history taking and a meticulous basic clinical examination. After that each participant answers the BDI II and SIS. Then they are aubjected to a resting HRV and Ewing's Battery of Tests for dysautonomia. Finally a blood sample was drawn for estimation of serum Il 6 levels and serum Lipid profile.

The results obtained by this explained methods were then statistically analysed.

Results

RESULTS

The data obtained from the above said methods were statistically analysed using SPSS software version 17, MS Office 2007 and EPI INFO-7 software

The correlation between individual parameters were analysed by Pearson's rank correlation .

Baseline Parameters:

Table 5. Baseline Parameters of Participants

PARAMETER	MEAN	SD
AGE	35.86	± 9.85
HR	74.37	± 4.08
SBP	128.04	± 4.30
DBP	81.22	± 3.00

In total 51 patients who have attempted suicide for the first time participated in the study out of which 26 are females and 25 are males. The mean age of the participants was 35.86±9.85. The baseline heart rate was 74.37± 4.08. The systolic blood pressure measured before all the procedure was 128.04 ± 4.30 and the diastolic blood pressure was 81.22± 3.00.

The mean and standard deviation of the collected data that was included for analysis is as shown in the following table.

Table 6. Mean and Standard Deviation (SD) of Study Parameters

S.No.	PARAMETER	MEAN±SD
1.	Suicide Intent Scale (SIS)	20.33±5.56
2.	Beck's Depression Inventory (BDI)	22.57±8.09
3.	Interleukin 6 (IL 6) in pg/ml	78.31±92.95
4.	Total Cholesterol (TC) in mg/dl	156.22±24.09
5.	Low Density Lipoprotein (LDL) in mg/dl	82.06±17.19
6.	Triglycerides (TGL) in mg/dl	146.80±27.49
7.	LF/HF ratio	1.59±0.94
8.	Posture ratio (30 : 15)	1.07±0.07
9.	Valsalva ratio (VR)	1.17±0.12
10.	Expiration-Inspiration ratio (E/I)	1.11±0.06
11.	Orthostatic Standing Test Systolic Blood Pressure (OST SBP_1) in mmHg	2.24±6.43
12.	Isometric Hand Grip Test Diastolic Blood Pressure (IGHT DBP_1) in mmHg	14.12±3.74

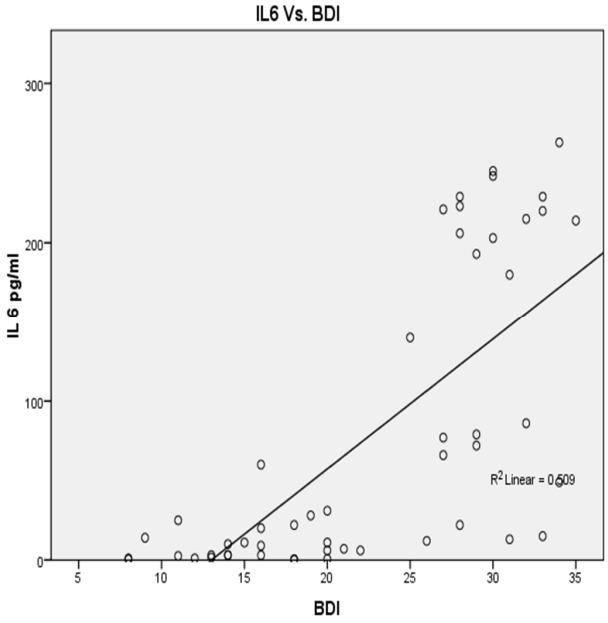
The above parameters were taken for analysis statistically. The correlation of IL 6,TC,LDL,TGL,LF/HF,30:15,VR,E/I,OST SBP_1,IHGT DBP_1 with both BDI and SIS scores have been calculated by Pearson's method.

Relationship between Serum IL 6 levels with BDI and SIS scores :

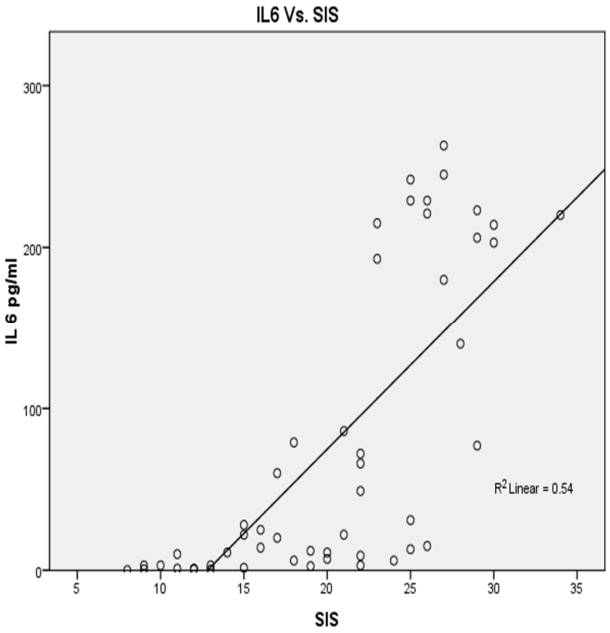
Table 7 : Correlation of IL 6 with BDI and SIS

	Mean ± SD	**Pearson's correlation**	
IL 6 (pg/ml)	78.31±92.947	**IL 6 vs. BDI**	**IL 6 vs. SIS**
BDI	22.57 ±8.090	r=0.713	r=0.753
SIS	20.33±6.563	p=0.000*	p=0.000*
*p Value Significant at the level <0.05			

The mean of the Serum interleukin 6 levels in the serum samples tested is 78.31±92.947. The mean of the scores obtained in the Beck's Depression Inventory is 22.57±8.090 and the mean of SIS is 20.33±6.563. When analysed using Pearson's correlation the r value is 0.713 and 0.735 for BDI and SIS respectively. This is confirmed by the Spearman's rho correlation which gives the r value of 0.753 and 0.795 for BDI and SIS respectively. On calculating the statistical significance at the level of $p< 0.05$ a high level of significance was got for both the parameters. Thus serum IL 6 levels are strongly correlated to the BDI and SIS scores in the positive direction.



Graph 2. Scatter plot of BDI score with IL 6



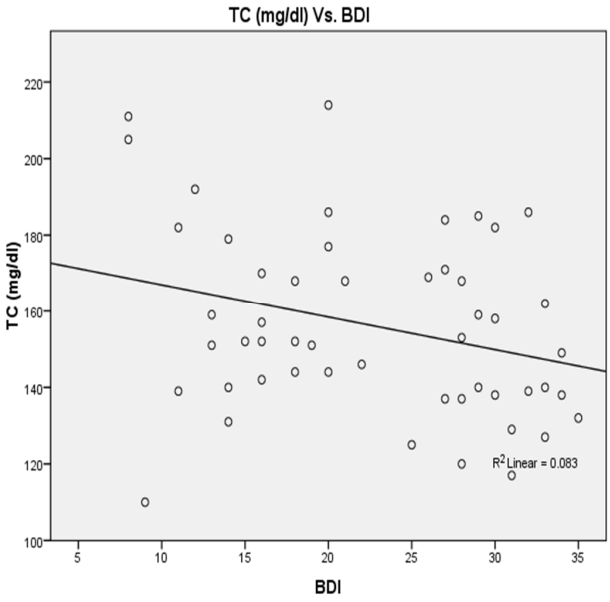
Graph 3. Scatter plot of SIS score with IL 6

Relationship of Serum Total Cholesterol levels with BDI and SIS:

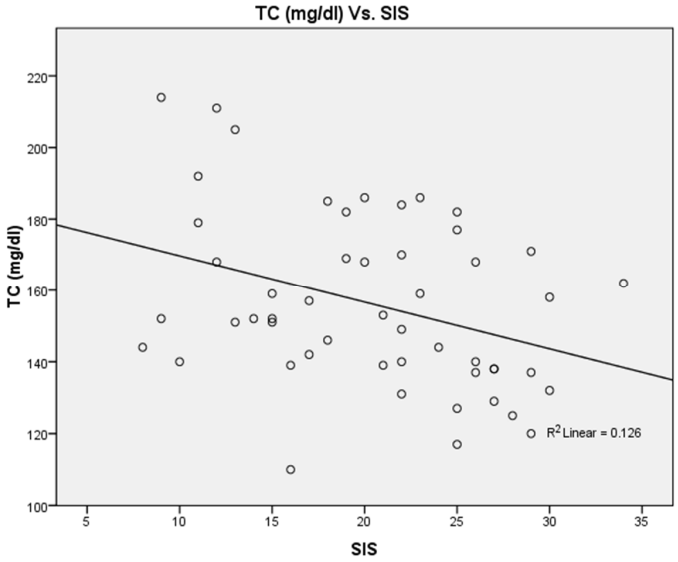
Table 8 : Correlation of TC with BDI and SIS

	Mean ± SD	**Pearson's correlation**	
TC (mg/dl)	156.22±24.097	**TC vs. BDI**	**TC vs. SIS**
BDI	22.57 ±8.090	r= -0.287	r= -0.355
SIS	20.33±6.563	p=0.041*	p=0.011*
*p Value Significant at the level <0.05			

The mean of Serum levels of total cholesterol measured in mg/dl was 156.22±24.097. This shows a weak downhill relationship with BDI and SIS scores with r value of -0.287 and -0.355 respectively. The p value gives a statistical significance in both the cases.



Graph 4. Scatter plot of BDI score with TC



Graph 5. Scatter plot of SIS score with TC

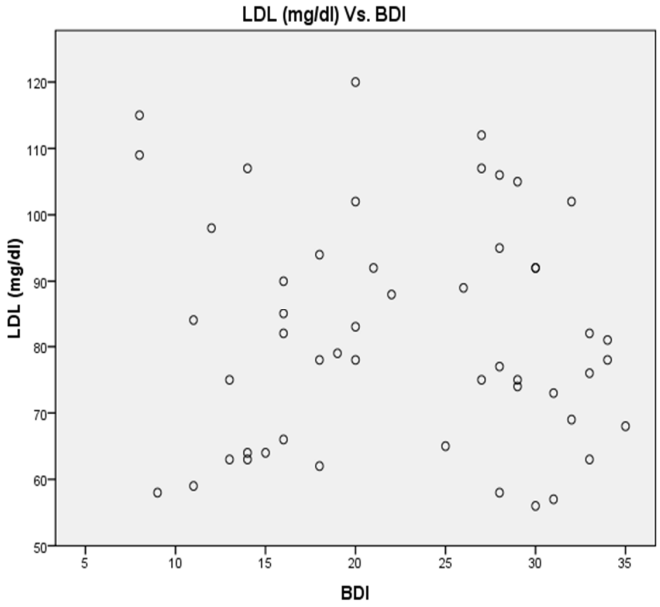
Relationship of serum LDL levels with BDI and SIS

Table 9 : Correlation of LDL with BDI and SIS

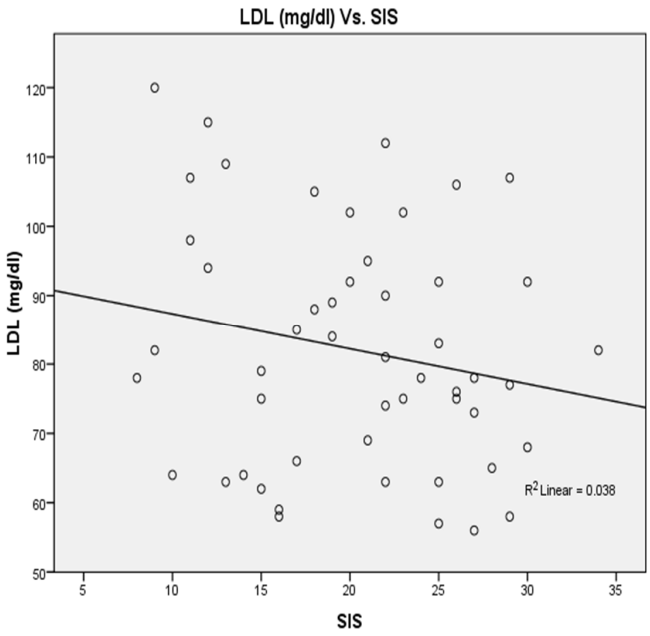
	Mean ± SD	**Pearson's correlation**	
LDL (mg/dl)	82.06±17.195	**LDL vs. BDI**	**LDL vs. SIS**
BDI	22.57 ±8.090	r= -0.074	r= -0.195
SIS	20.33±6.563	p=0.605*	p=0.171*
*p Value Significant at the level <0.05			

The mean of serum levels of LDL cholesterol measured in mg/dl is 82.06 ±17.195. This when correlated with that of BDI and SIS scores shows a very weak downhill linear relationship with r value of -0.074 and -0.195 respectively.

This is very much nearer to zero. And also the p value is >0.05 in both cases. So the relationship of LDL levels with both parameters is not statistically significant.



Graph 6. Scatter plot of BDI score with LDL



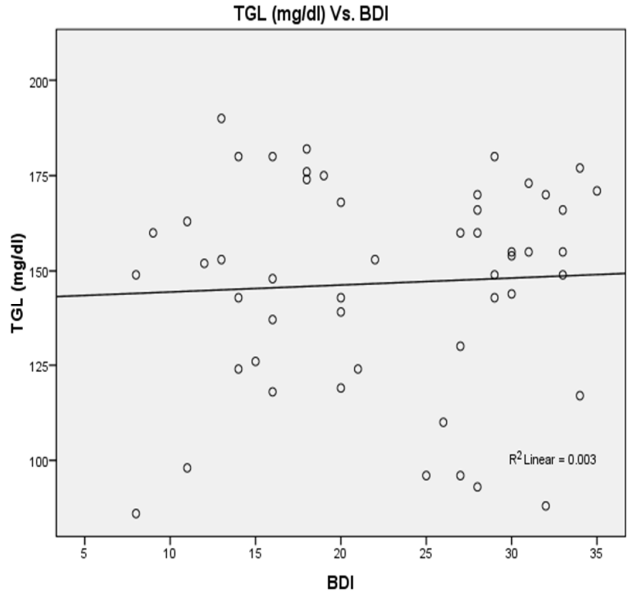
Graph 7. Scatter plot of SIS score with LDL

Relationship of serum triglyceride levels with BDI and SIS:

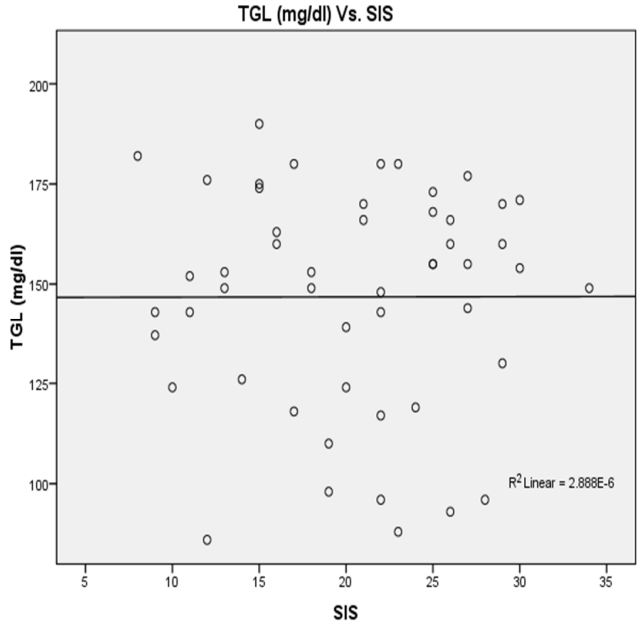
Table 10 : Correlation of TGL with BDI and SIS

	Mean ± SD	**Pearson's correlation**	
TGL (mg/dl)	146.80±27.497	**TGL vs. BDI**	**TGL vs. SIS**
BDI	22.57 ±8.090	r= 0.054	r=0.002
SIS	20.33±6.563	p=0.708	p=0.991
*p Value Significant at the level <0.05			

The mean of serum TGL levels in mg/dl was 146.80±27.497. when this was analysed for correlation with the mean of BDI scores the r value is 0.054 which very close to zero. Also the r value for mean of SIS scores is 0.002 which is nearly equal to zero. With this when statistical significance is calculated for these parameters low level of significance is observed as evidenced by p value > 0.05.



Graph 8. Scatter plot of BDI score with TGL



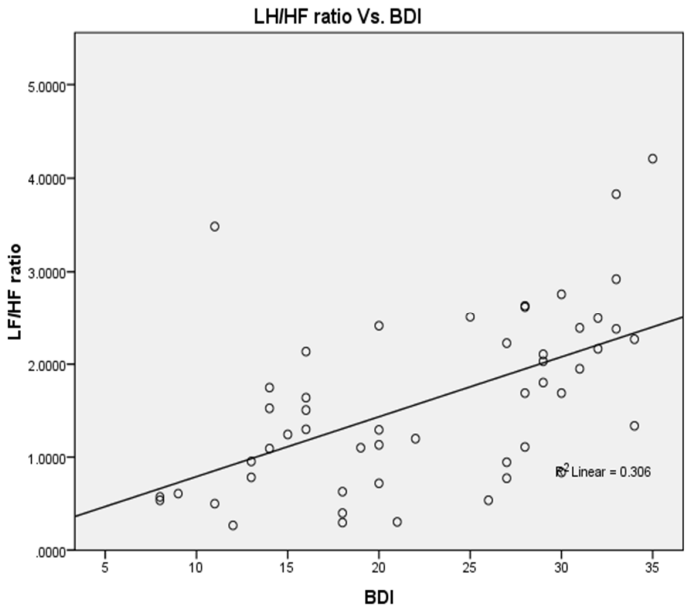
Graph 9. Scatter plot of SIS score with TGL

Relationship of LF/ HF ratio with BDI and SIS:

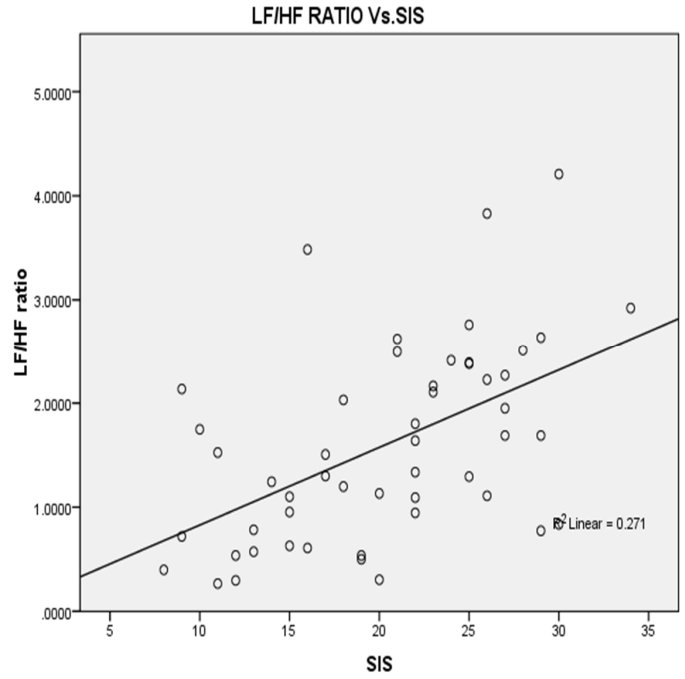
Table 11 : Correlation of LF/HF with BDI and SIS

	Mean ± SD	**Pearson's correlation**	
LF/HF	1.59±0.94	**LF/HF vs. BDI**	**LF/HF vs. SIS**
BDI	22.57 ±8.090	r= 0.553	r=0.52
SIS	20.33±6.563	p=0.000*	p=0.000*
*p Value Significant at the level <0.05			

The mean of LF/HF ratio which is approximately 1.6 ±0.9 has a moderately positive linear relationship in the uphill direction with both BDI and SIS scores as evidenced by r value of 0.5. This also predicts a high level of statistical significance with a p value of 0.000 which is <0.05.



Graph 10. Scatter plot of BDI score with LF/HF



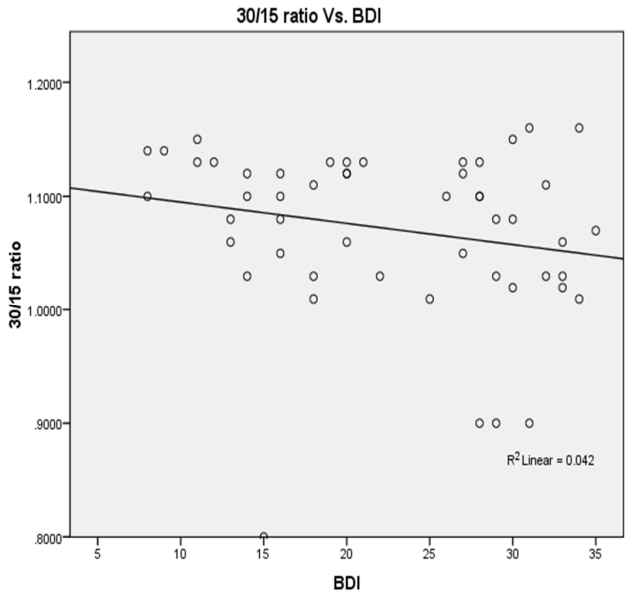
Graph 11. Scatter plot of SIS score with LF/HF

Relationship of posture ratio with BDI and SIS:

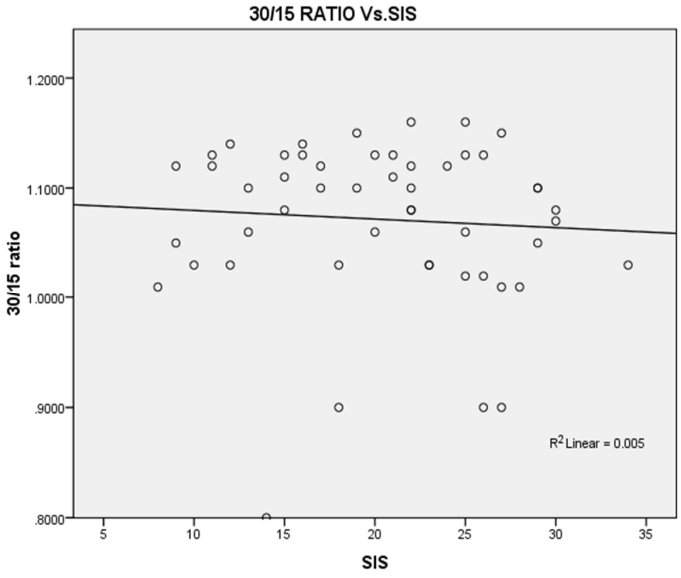
Table 12 : Correlation of 30/15 ratio with BDI and SIS

	Mean ± SD	**Pearson's correlation**	
30/15	1.071±0.07	**30/15 vs. BDI**	**30/15 vs. SIS**
BDI	22.57 ±8.090	r= -0.205	r= -0.07
SIS	20.33±6.563	p=0.15	p=0.627
*p Value Significant at the level <0.05			

The mean of 30:15 ratio is 1.07±0.07 when correlated with mean of scores of BDI and SIS the r value is -0.205 and -0.07 respectively. This denotes that the two parameters are negatively correlated to 30:15 ratio. Also there is no statistical significance as the p value is >0.05 in both these cases..



Graph 12. Scatter plot of BDI score with 30/15



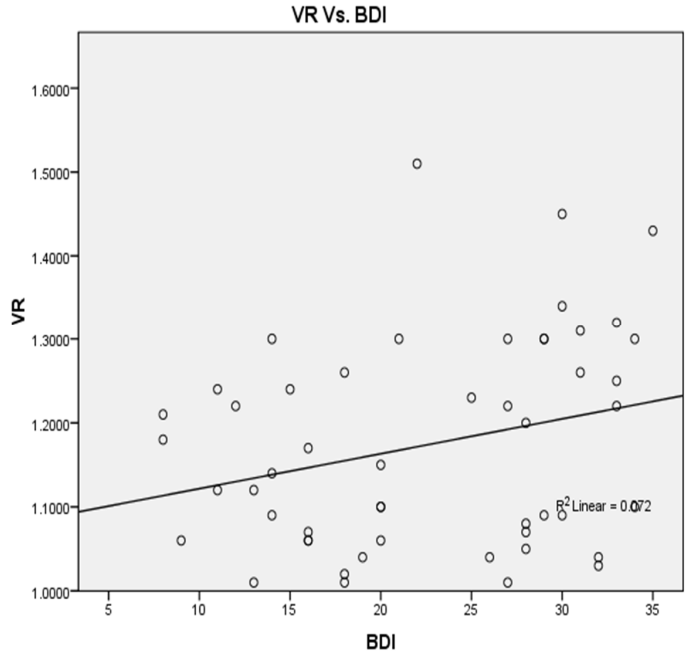
Graph 13. Scatter plot of SIS score with 30/15

Relationship of valsalva ratio with BDI and SIS:

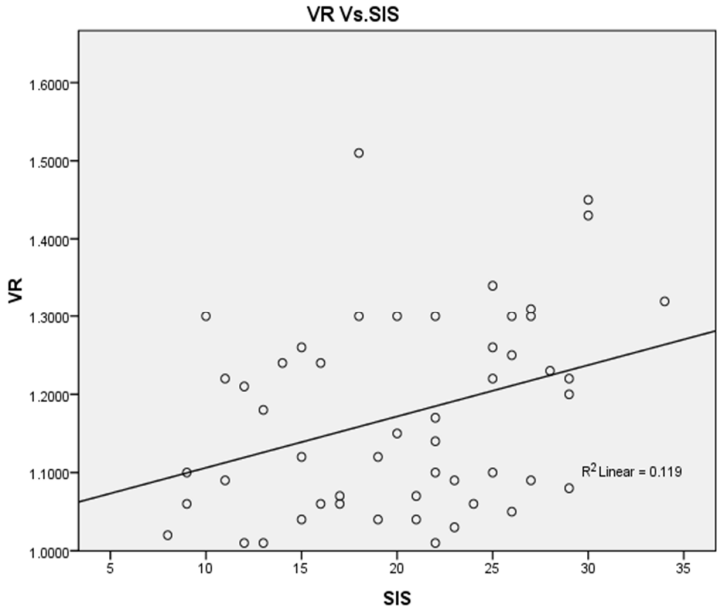
Table 13 : Correlation of VR with BDI and SIS

	Mean ± SD	**Pearson's correlation**	
VR	1.17±0.12	**30/15 vs. BDI**	**30/15 vs. SIS**
BDI	22.57 ±8.090	r=-0.269	r= -0.345
SIS	20.33±6.563	p=0.056	p=0.013*
*p Value Significant at the level <0.05			

The mean of valsalva ratio is 1.17±0.12 when subjected to correlation test it shows a weak uphill relationship with BDI and SIS with respective r values of 0.269 and 0.345.s. The p value also does not show a statistical significance with BDI. In contrast the p value of SIS is 0.013 which shows a high levels of statistical significance.



Graph 14. Scatter plot of BDI score with VR



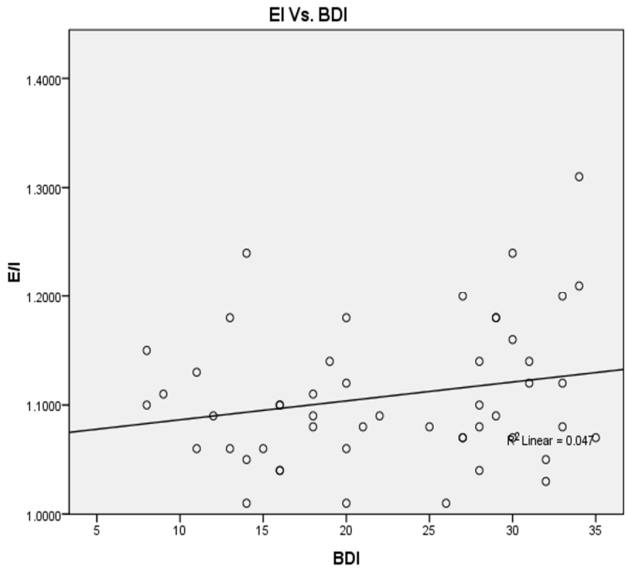
Graph 15. Scatter plot of SIS score with VR

Relationship of E/I ratio with BDI and SIS:

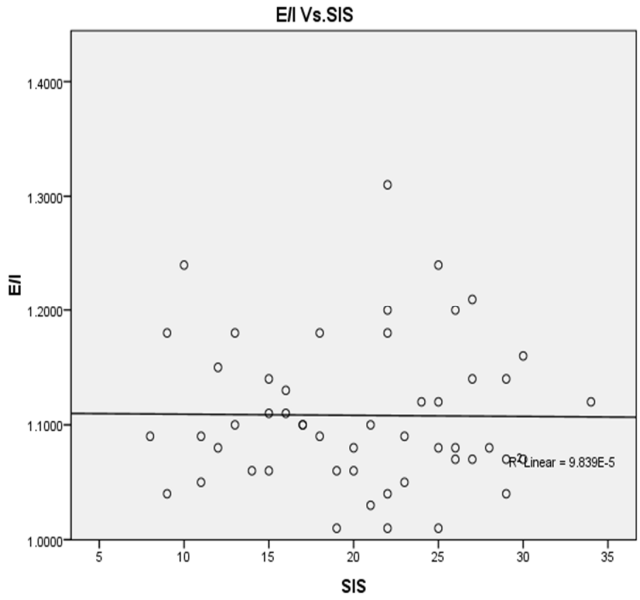
Table 14 : Correlation of E/I ratio with BDI and SIS

	Mean ± SD	**Pearson's correlation**	
E/I ratio	1.10±0.06	**E/I vs. BDI**	**E/I vs. SIS**
BDI	22.57 ±8.090	r=0.217	r= -0.12
SIS	20.33±6.563	p=0.126	p=0.945
*p Value Significant at the level <0.05			

The mean of E/I ratio (1.10±0.06) when analysed for correlation with the mean of scores of BDI and SIS gives a weak downhill linear relationship with respective r values of -0.217 and-0.012 .also as evidenced by p>0.05 the level of statistical significance is low.



Graph 16. Scatter plot of BDI score with E/I



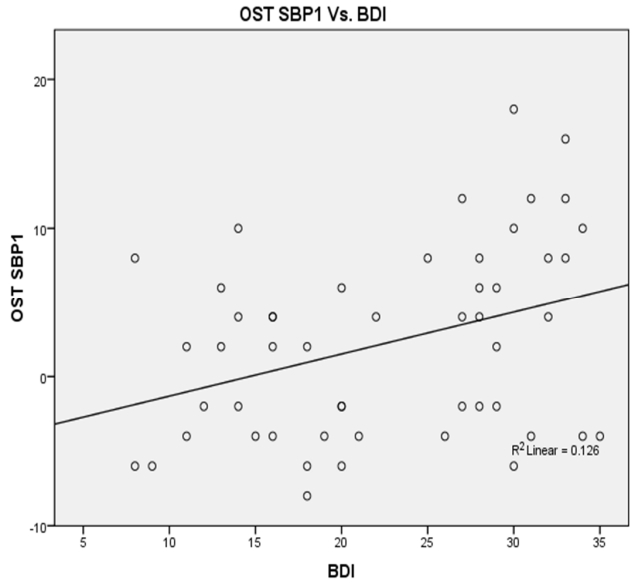
Graph 17. Scatter plot of SIS score with E/I

Relationship of OST SBP_1 with BDI and SIS:

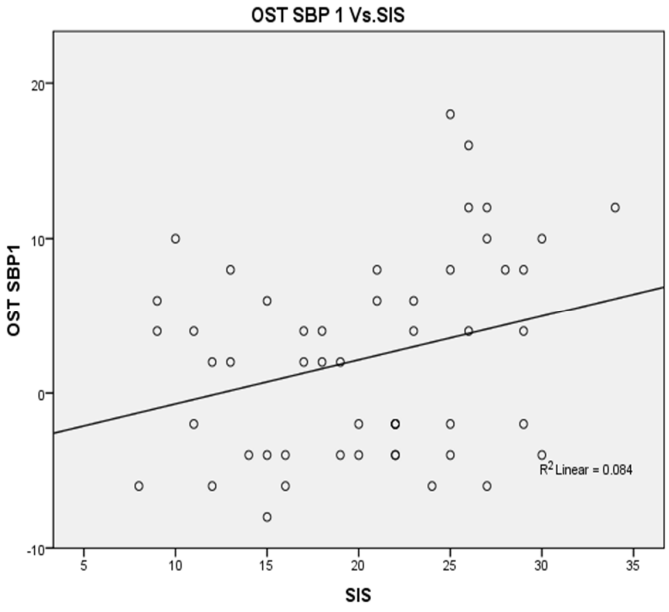
Table 15 : Correlation of OST SBP_1 with BDI and SIS

	Mean ± SD	Pearson's correlation	
OST SBP_1	2.24±6.43	**OST SBP_1 vs. BDI**	**OST SBP_1 vs. SIS**
BDI	22.57 ±8.090	r=0.355	r=0.29
SIS	20.33±6.563	p=0.011*	p=0.039*
*p Value Significant at the level <0.05			

The mean of decrease in systolic BP as measured by orthostatic standing test is 2.24±6.433 which when correlated with mean of BDI and SIS scores the r values are of 0.335 and 0.29 respectively. High levels of statistical significance is evidenced by p value <0.05.



Graph 18. Scatter plot of BDI score with OST SBP_1



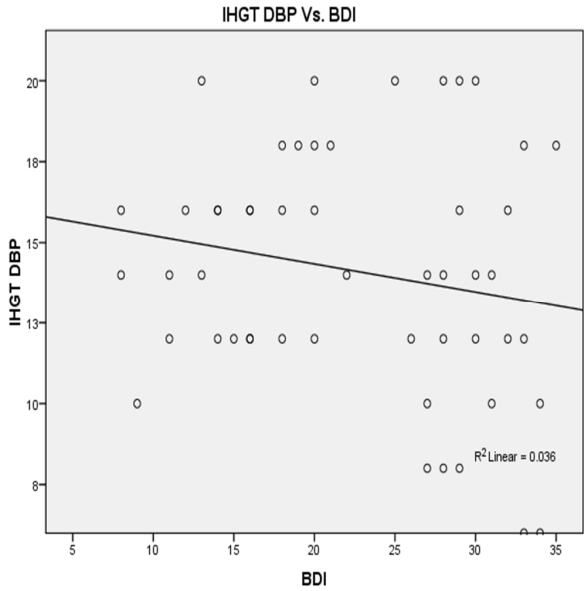
Graph 19. Scatter plot of SIS score with OST SBP_1

Relationship of IHGT DBP_1 with BDI and SIS:

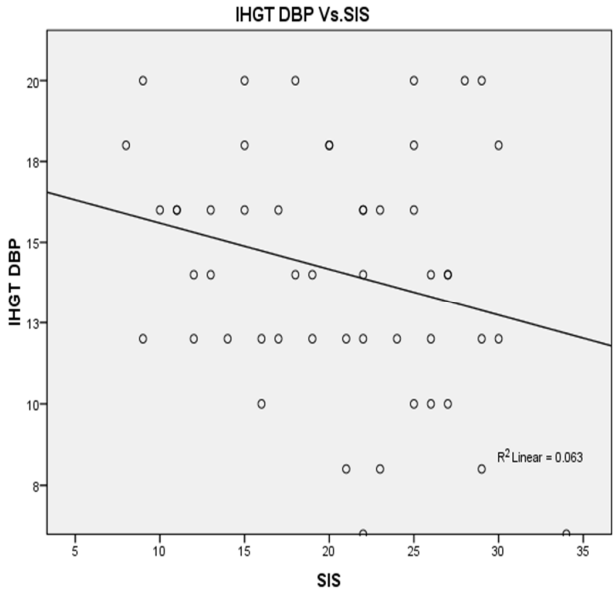
Table 16 : Correlation of IHGT DBP_1 with BDI and SIS

	Mean ± SD	Pearson's correlation	
IHGT DBP_1	14.12±3.74	**IHGT DBP_1 vs. BDI**	**IHGT DBP_1 vs. SIS**
BDI	22.57 ±8.090	r= -0.189	r= -0.251
SIS	20.33±6.563	p=0.185	p=0.075
*p Value Significant at the level <0.05			

The mean of increase in Diastolic BP as measured by the Isometric Hand Grip Test is 14.12±3.74. When analysed for correlation with the mean of BDI and SIS scores the r value is -0.189 and -0.251.this denotes a weak negative correlation of IHGT DBP with both BDI and SIS. As the p value is >0.05 the level of significance is low.



Graph 20 . Scatter plot of BDI score with IHGT DBP_1



Graph 21. Scatter plot of BDI score with IHGT DBP_1

The r value for the analysed parameters and the p value calculated at the levels of significance $p<0.05$ are tabulated as follows.

Table 17. Comparison of r and p value of IL 6, TC, LDL & TGL with BDI & SIS

		IL 6 (pg/ml)	TC (mg/dl)	LDL (mg/dl)	TGL (mg/dl)
SIS	r Value	0.735	-0.355	-0.195	0.002
	p Value	0.000*	0.011*	0.171	0.991
BDI	r Value	0.713	-0.287	-0.074	0.054
	p Value	0.000*	0.041*	0.605	0.708

Table 18. Comparison of r and p value of Ewing's battery with BDI & SIS

		LF/HF (ratio)	30:15 (ratio)	VR (ratio)	E/I (ratio)	OST SBP_1	IHGT DBP_1
SIS	r Value	0.52	-0.07	0.345	-0.012	0.29	-0.251
	p Value	0.000*	0.627	0.013*	0.945	0.039*	0.0756
BDI	r Value	0.553	-0.205	0.269	-0.217	0.355	-0.189
	p Value	0.000*	0.15	0.056	0.126	0.011*	0.185

Thus from the above results the inference is

1. Serum Interleukin 6 levels and LF/HF ratio show a positive correlation with BDI and SIS scores with high levels of statistical significance.
2. OST SBP shows a statistically significant weak positive correlation with BDI and SIS scores.
3. The Serum Total cholesterol levels show a weak negative correlation to BDI scores which is statistically significant and also with SIS scores but with high levels of statistical significance.
4. The Valsalva ratio shows a weak positive correlation with both BDI and SIS scores which is not so significant with the former but highly significant with later in statistical grounds.
5. The other parameters are not significantly correlated as evidenced by the p value.

Discussion

DISCUSSION

The present study was done to study the role of cytokine markers and autonomic function tests in identifying depression in persons attempting suicide.

This was done by

1. detecting the Interleukin 6 in the serum of study subjects
2. using Ewing's Battery of Autonomic function tests.

We have also attempted to figure out the mechanism by which the cytokines influence the depression scores by testing the lipid profile in the subjects.

High IL 6 and Suicide attempters:

J Isung et al[105] in 2014 have concluded in their study on endophenotypic determinants of suicide attempts, that neurotic personality traits like impulsivity and aggression are closely associated with attempting suicide. Neuroticsm is the cause for depression. Also there was high correlation of these personality traits with serum and csf IL 6 levels. This explains the neuroinflammation hypothesis for attempting suicide.

Lindqvist et al[106] have published yet another study which was concluded that there is a strong positive correlation between MADRS scores and CSF IL 6 levels in suicide attempters. Since there was a correlation of CSF IL 6 levels also with the 5-HIAA and HVA levels the authors have proposed that the mechanism by which IL 6 is involved is through alterations in the serotonergic and dopaminergic metabolism.

Licínia Ganança et al[107] have done a metaanalysis on cytokines in pathophysiology of suicide. A thorough search was done in the databases of Pubmed, Embase, Scopus and PsycINFO from 1980 through 2015. 22 articles were found to be directly assessing the link between cytokines and suicide. Of these 8 studies reported IL 6 as the frequently associated cytokine with suicide attempt.

One study done by Kim et al[108] compared blood levels of various cytokines between depressed patients who have attempted and not attempted suicide. They have found out that non suicidal depressed patients have higher levels of IL 6 than suicidal patients and controls. But however this study also supports the finding of elevated levels of IL 6 in depression.

Cytokines and Depression :

Berk et al[109] has done a literature review on the link between inflammation and clinical depression. The authors have discovered that the various symptom complexes of depression like sleep disturbances, altered appetite, vulnerability to stress, addiction etc. have a bidirectional relationship with the inflammatory processes that result in elevated cytokine levels.

Reichenberg et al[110] has done a experimental study by inducing immune response in healthy subjects by injecting endotoxin. When cognitive and emotional assessment was done in these subjects there was increased anxiety, depressed mood and memory disturbances which positively correlated with increased cytokine levels in response to the endotoxin.

The present study have also shows a significantly elevated levels of Serum IL 6 in suicide attempters who also show high scores of depression.

Lipid profile , depression and suicide:

Patra BN et al[111] studied the serum lipid profile in Indian patients suffering from depression.they found out that serum TC and LDL were significantly lower in patients with depressive episode when compared to normal controls. But there was no relationship between HDL levels and depression.

Anita B Kale et al[112] measured the lipid parameters in patients with endogenous depression and compared them with that of normal controls. In their study all parameters- TC,LDL, HDL,TGL and VLDL showed significantly low levels in the serum in comparison with the controls. Also there was a definite correlation of these parameters with BDI scores.

Malcom garland et al[113] studied Serum Total cholesterol levels in suicide attempters and compared them with normal controls and controls with psychiatric diagnosis but not have attempted suicide. There was low levels of TC in suicide attempters whereas very high levels in the psychiatric controls compared with normal controls.

Luis G.Almeida-Montes et al[114] studied the realationship of lipid parameters and severity of depression and suicide attempts. They found that there is no apparent relation between the lipid profile with the other two parameters. Total cholesterol and the lipoprotein components were found to be equal to that of

the controls while the study population showed significant alterations in depression scores and serotonin levels.

This current study shows that the lipid parameters other than Total Cholesterol does not show any significant relation either with the BDI or SIS scores.

Misato Hashizume and Mashiko Mihara[115] in their review on effects of IL 6 in lipid metabolism have concluded that IL 6 stimulates uptake of lipids into tissues by upregulating VLDL receptors, decreases cholesterol synthesis by the liver and increases breakdown of lipids in liver and adipose tissue.

So when we hypothesized this study we expected a low lipid parameters that correlated strongly with the BDI and SIS scores if IL 6 levels are going to be high and strongly correlating with both these parameters. As it is not so we have to think about some other mechanism through which IL 6 influences depression and suicidal behavior.

HRV and suicide:

Wilson ST et al[116] compared HRV between suicide attempters and non attempters during a Trier Social Stress Test and found that the attempters show low cumulative HF in HRV than the non attempters. This shows that the suicide attempters have decreased capacity to handle stress and the response to stress.

Forkmann T et al [117]studied HRV in normal persons and scored suicidal ideation after controlling for depression and found that persons with high suicidal ideation have low vagal tone in resting state.

HRV and depression:

Nahshoni E et al[118] studied the HRV in three groups of patients viz one with depression and no physical illness; second is a group of heart transplant patients with no mental illness and third is a group of healthy controls. The authors found lower Heart Rate Variability in both patient groups when compared to normal subjects. But there was a still more reduction of vagal modulation of heart rate in depressed patients.

Andrew H Kemp et al[119] analysed the HRV in patients with MDD both melancholics and non melancholics in comparison with normal controls. The melancholics showed an increase in heart rate and a reduction in RMSSD, PCSDI and HF in coparison with normals but non melancholics were comparable to normal subjects.

On analyzing HRV both resting and during manuoveurs in the present study all variables other than the LF/HF ratio shows weak or no correlation with depression or suicide intent scores. This shows that sympathovagal imbalance is definitely present in suicide attempters with depression. But the negative findings in the other parameters must be explored further. Due to time constraints only a short term HRV was recorded. If we go for a holter monitoring analysis of HRV more accurate picture could be obtained.

Condusion

CONCLUSION

The conclusions derived from this study are

- Serum interleukin 6 levels are elevated in suicide attempters.
- The serum IL 6 levels are elevated in relation to the depression scores (BDI) of suicide attempters.
- The depression scores increase as the serum total cholesterol levels decrease in suicide attempters.
- The LF/HF ratio increases as the depression scores increase indicating a sympathovagal imbalance in suicide attempters.

So we could conclude from the present study that first time suicide attempters should be screened for depression with complete psychosocial assessment and a reliable instrument like BDI to detect even mild clinical depression. Also we should explore the presence of biological factors like neuroinflammation, low cholesterol and autonomic dysregulation in these group of patients which could lead to the possible interventions that could be made to prevent further attempts.

Limitations of the study

LIMITATIONS OF THE STUDY

- Considering the limitations in time and cost of the investigations the sample size was very much smaller.
- The findings were not compared with controls.
- The cut off limits for Lipid Profile has not been set at the start of the study.
- Measuring Interleukin 6 levels in the CSF would be a more reliable parameter than serum IL 6 levels.
- Measuring catecholamines and their metabolites would be a better indicator of autonomic functions than HRV.

If these limitations are overcome and if each of these parameters are studied separately in an elaborate manner in these subset of patients we could get a reliable leading pathway towards a better understanding of the complex pathophysiology of suicide.

Summary

SUMMARY

A study was conducted on persons who do not have any past or present history of psychiatric illness and had attempted suicide for the first time in any modality to measure the Serum Interleukin 6 levels and fasting lipid profile and to evaluate the cardiac autonomic functioning.

Fifty one persons in the age group of 20-40 years in both genders participated in the study. The intention to die was scored with Beck's Suicide Intent Scale(SIS) and they were screened for Depression using Beck's Depression Inventory II (BDI II). The participants underwent the Ewing's Battery of tests for autonomic functioning. The Serum IL 6 and Lipid profile was measured in a fasting serum sample.

There was significant high values of serum IL 6 levels and the serum Total Cholesterol (TC) levels were lower. There was significant increase in LF/HF ratio denoting a sympathovagal imbalance in these subjects. All these observations significantly correlated with SIS and BDI scores.

So serum IL 6 , TC and LF/HF ratio can be regarded as biological markers of depression in suicidal patients.

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Annexures

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.K.Sowmiya
Post Graduate in M.D.Physiology
Institute of Physiology and Experimental Medicine
Madras Medical College
Chennai 600 003

Dear Dr.K.Sowmiya,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY OF AUTONOMIC DYSFUNCTIONS AND INTERLEUKIN-6 MEDIATED CHANGES IN SERUM LIPID PROFILE AS BIO-MARKER OF DEPRESSION IN ATTEMPTED SUICIDE PATIENTS" NO. 21062016.**

The following members of Ethics Committee were present in the meeting hold on **07.06.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.Isaac Christian Moses,MD.Ph.D.Dean(FAC)MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	:MemberSecretary
4.Prof.B.Vasanthi,MD., Prof.of Pharmacology.,MMC,Ch-3	: Member
5.Prof.P.Raghumani,MS, Prof. of Surgery,RGGGH,Ch-3	: Member
6.Prof.Baby Vasumathi, Director, Inst. of O&G,Ch-8	: Member
7.Prof.K.Ramadevi,MD, Director,Inst.of Bio-Chem,MMC,Ch-3	: Member
8.Prof.M.Saraswathi,MD.,Director, Inst.of Path,MMC,Ch-3	: Member
9.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
10.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
11.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary - Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

INFORMED CONSENT FORM

Title of the study : **"A Study of autonomic dysfunctions and Interleukin-6 mediated changes in Serum Lipid Profile as Bio-Marker of Depression in Attempted suicide patients"**

Name of the Participant:

Name of the Principal Investigator: Dr.K.SOWMIYA

Name of the Institution: Institute of Physiology and Experimental Medicine, Madras Medical College and Govt. General Hospital, Chennai – 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in "A Study of autonomic dysfunctions and Interleukin-6 mediated changes in Serum Lipid Profile as Bio-Marker of Depression in Attempted suicide patients"

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past_____month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.
12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

13. I have understand that my identity will be kept confidential if my data are publicly presented.

14. I have had my questions answered to my satisfaction.

15. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signingthis consent form I attest that the information given in this document has been clearly explained to meand understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

ஆய்வு பங்கேற்பாளர் ஒப்புதல் படிவம்

ஆராய்ச்சியின் விவரம் : தற்கொலைக்கு முயன்றோர் இடையே, இருதய தானியங்கி நரம்பு மண்டல மாறுபாடுகள் மற்றும் இரத்தத்தில் இன்டர்லூகின்-6 உடனான கொழுப்பு சத்தின் அளவினை மன அழுத்தத்திற்காக உயிர் குறியீடாக ஆய்வு செய்தல்.

ஆராய்ச்சி மையம் :

நோயாளியின் பெயர் : நோயாளியின் வயது :

பதிவு எண். :

நோயாளி கீழ்கண்டவற்றுள் கட்டங்களை (✓) செய்யவும்

1 மேற்குறிப்பிட்டுள்ள ஆராய்ச்சியின் நோக்கத்தையும் பயனையும் முழுவதுமாக புரிந்துகொண்டேன். மேலும் எனது அனைத்து சந்தேகங்களையும் கேட்டு அதற்கான விளக்கங்களையும் தெளிவுப்படுத்திக்கொண்டேன். ☐

2 மேலும் இந்த ஆராய்ச்சிக்கு எனது சொந்த விருப்பத்தின் பேரில் பங்கேற்கிறேன் என்றும், மேலும் எந்த நேரத்திலும் எவ்வித முன்னறிவிப்பின்றி இந்த ஆராய்ச்சியிலிருந்து விலக முழுமையான உரிமை உள்ளதையும், இதற்கு எவ்வித சட்ட பிணைப்பும் இல்லை என்பதையும் அறிவேன். ☐

3 ஆராய்ச்சியாளரோ, ஆராய்ச்சி உதவியாளரோ, ஆராய்ச்சி உபயத்தாரோ, ஆராய்ச்சி பேராசிரியரோ, ஒழுங்குநெறி செயற்குழு உறுப்பினர்களோ எப்போது வேண்டுமானாலும் எனது அனுமதியின்றி எனது உள்நோயாளி பதிவுகளை இந்த ஆராய்ச்சிக்காகவோ அல்லது எதிர்கால பிற ஆராய்ச்சிகளுக்காகவோ பயன்படுத்திக்கொள்ளலாம் என்றும், மேலும் இந்த நிபந்தனை நான் இவ்வாராய்ச்சியிலிருந்து விலகினாலும் தகும் என்றும் ஒப்புக்கொள்கிறேன். ஆயினும் எனது அடையாளம் சம்பந்தப்பட்ட எந்த பதிவுகளும் (சட்டப்பூர்வமான தேவைகள் தவிர) வெளியிடப்படமாட்டாது என்ற உறுதிமொழியின் பெயரில் இந்த ஆராய்ச்சியிலிருந்து கிடைக்கப்பெறும் முடிவுகளை வெளியிட மறுப்பு தெரிவிக்கமாட்டேன் என்று உறுதியளிக்கின்றேன். ☐

4 இந்த ஆராய்ச்சிக்கு நான் முழுமனதுடன் சம்மதிக்கின்றேன் என்றும், மேலும் ஆராய்ச்சிக் குழுவினர் எனக்கு அளிக்கும் அறிவுரைகளை தவறாது பின்பற்றுவேன் என்றும், இந்த ஆராய்ச்சி காலம் முழுவதும் எனது உடல் நிலையில் ஏதேனும் மாற்றமோ அல்லது எதிர்பாராத பாதகமான விளைவோ ஏற்படுமாயின் உடனடியாக ஆராய்ச்சி குழுவினரை அணுகுவேன் என்றும் உறுதியளிக்கின்றேன். ☐

5 இந்த ஆராய்ச்சிக்குத் தேவைப்படும் அனைத்து மருத்துவப் பரிசோதனைகளுக்கும் ஒத்துழைப்பு தருவேன் என்று உறுதியளிக்கின்றேன். ☐

6 இந்த ஆராய்ச்சிக்கு யாருடைய வற்புறுத்தலுமின்றி எனது சொந்த விருப்பத்தின் பேரிலும், சுய அறிவுடனும், முழுமனதுடனும் சம்மதிக்கின்றேன் என்று இதன்மூலம் ஒப்புக்கொள்கிறேன். ☐

நோயாளியின் கையொப்பம் / பெருவிரல் கைரேகை ஆராய்ச்சியாளரின் கையொப்பம்

இடம் : தேதி :

PROFORMA

Name: Age/ Sex: IP/OP No.:

Marital status: Address:

Education: Occupation:

Contact phone No.

No. of Suicide Attempts:

Mode of attempt:

History of fever, injury:

History of recent infections:

History of any chronic illness (specify):

History of chronic drug intake (specify):

History of Substance abuse :

Investigations:

Serum Interleukin 6		pg/ml
Serum Lipid Profile	Total cholesterol	mg/dl
	HDL	mg/dl
	LDL	mg/dl
	VLDL	mg/dl
	TGL	mg/dl

EXAMINATION

General examination:

Temperature:

Pulse rate:

Blood pressure:

Systemic examination:

Cardiovascular system:

Respiratory system:

Gastrointestinal system:

Central nervous system:

Mental Status Examination:

BDI II score: SIS score:

BECK'S DEPRESSION INVENTORY II

Roche | **Beck Depression Inventory** | **Baseline**

V 0477 | CRTN: _____ CRF number: _____ | Page 14 | patient inits: _____

BDI-II | Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness

0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism

0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure

0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure

0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings

0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. Punishment Feelings

0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike

0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness

0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes

0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying

0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

11. Agitation

0 I am no more restless or wound up than usual.

1 I feel more restless or wound up than usual.

2 I am so restless or agitated that it's hard to stay still.

3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.

1 I am less interested in other people or things than before.

2 I have lost most of my interest in other people or things.

3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.

1 I find it more difficult to make decisions than usual.

2 I have much greater difficulty in making decisions than I used to.

3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.

1 I don't consider myself as worthwhile and useful as I used to.

2 I feel more worthless as compared to other people.

3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.

1 I have less energy than I used to have.

2 I don't have enough energy to do very much.

3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.

1a I sleep somewhat more than usual.

1b I sleep somewhat less than usual.

2a I sleep a lot more than usual.

2b I sleep a lot less than usual.

3a I sleep most of the day.

3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.

1 I am more irritable than usual.

2 I am much more irritable than usual.

3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.

1b My appetite is somewhat greater than usual.

2a My appetite is much less than before.

2b My appetite is much greater than usual.

3a I have no appetite at all.

3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.

1 I can't concentrate as well as usual.

2 It's hard to keep my mind on anything for very long.

3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.

1 I get more tired or fatigued more easily than usual.

2 I am too tired or fatigued to do a lot of the things I used to do.

3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.

1 I am less interested in sex than I used to be.

2 I am much less interested in sex now.

3 I have lost interest in sex completely.

BECK'S SUICIDE INTENT SCALE

1. Isolation
    1. Somebody present
    2. Somebody nearby, or in visual or vocal contact
    3. No one nearby or in visual or vocal contact

2. Timing
    1. Intervention is probable
    2. Intervention is not likely
    3. Intervention is highly unlikely

3. Precautions against discovery/intervention-
    1. No precautions
    2. Passive precautions (as avoiding other but doing nothing to prevent their intervention; alone in room with unlocked door)
    3. Active precautions (as locked door)

4. Acting to get help during/after attempt
    1. Notified potential helper regarding attempt
    2. Contacted but did not specifically notify potential helper regarding attempt
    3. Did not contact or notify potential helper

5. Final acts in anticipation of death (will, gifts, insurance)
    1. None
    2. Thought about or made some arrangements
    3. Made definite plans or completed arrangements

6. Active preparation for attempt
    1. None
    2. Minimal to moderate
    3. Extensive

7. Suicide Note
    1. Absence of note
    2. Note written, but torn up; note thought about
    3. Presence of note

8. Overt communication of intent before the attempt
    1. None
    2. Equivocal communication
    3. Unequivocal communication

Self Report

9. Alleged purpose of attempt
    1. To manipulate environment, get attention, get revenge
    2. Components of above and below
    3. To escape, surcease, solve problems

10. Expectations of fatality
    1. Thought that death was unlikely
    2. Thought that death was possible but not probable
    3. Thought that death was probable or certain

11. Conception of method's lethality
    1. Did less to self than s/he thought would be lethal
    2. Wasn't sure if what s/he did would be lethal
    3. Equaled or exceeded what s/he thought would be lethal

12. Seriousness of attempt
    1. Did no seriously attempt to end life
    2. Uncertain about seriousness to end life
    3. Seriously attempted to end life

13. Attitude toward living/dying
    1. Did not want to die
    2. Components of above and below
    3. Wanted to die

14. Conception of medical rescuability
    1. Thought that death would be unlikely if he received medical attention
    2. Was uncertain whether death could be averted by medical attention
    3. Was certain of death even if he received medical attention

15. Degree of premeditation
    1. None; impulsive
    2. Suicide contemplated for three hours of less prior to attempt
    3. Suicide contemplated for more than three hours prior to attempt

Other Aspects (Not included in total score)

16. Reaction to attempt
    1. Sorry it was made; feels foolish; ashamed
    2. Accepts both attempt and failure
    3. Regrets failure of attempt

17. Visualization of death
    1. Life after death, reunion with descendants
    2. Never-ending sleep, darkness, end of things
    3. No conceptions of or thoughts about death

18. Number of previous attempts
    1. None
    2. One or two
    3. Three or more

19. Relationship between alcohol intake and attempt
    1. Some alcohol intake prior to but not related to attempt; reportedly not enough to impair judgment, reality testing
    2. Enough alcohol intake to impair judgment; reality testing and diminish Responsibility
    3. Intentional intake of alcohol in order to facilitate implementation of attempt

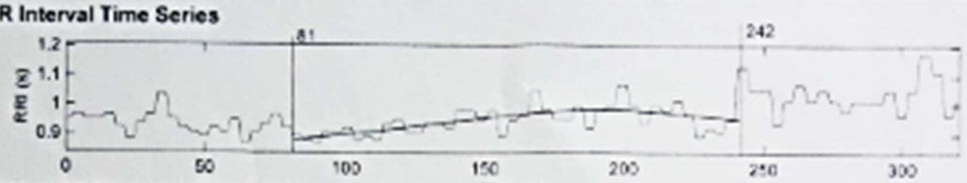
20. Relationship between drug intake and attempt
    1. Some drug intake prior to but not related to attempt; reportedly not enough to impair judgment, reality testing
    2. Enough drug intake to impair judgment; reality testing and diminish responsibility
    3. Intentional intake of drug in order to facilitate implementation of attempt

HRV ANALYSIS REPORT

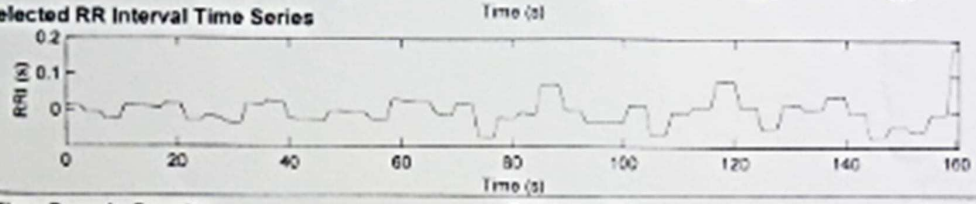
Heart Rate Variability Analysis

hrv201606
Page 1/1

RR Interval Time Series



Selected RR Interval Time Series

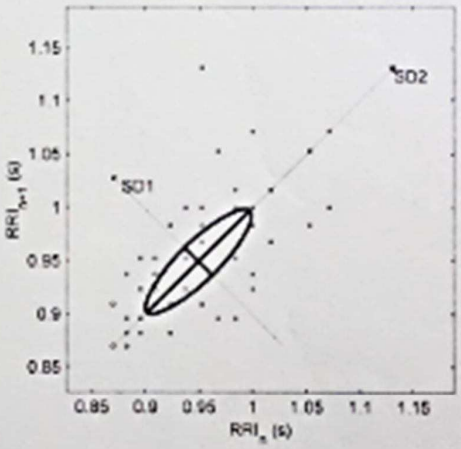


Time Domain Results

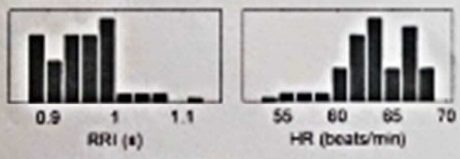
Variable	Units	Value
Statistical Measures		
Mean RR*	(s)	0.949
STD	(s)	0.038
Mean HR*	(1/min)	63.38
STD	(1/min)	2.47
RMSSD	(ms)	25.8
NN50	(count)	14
pNN50	(%)	8.3
Geometric Measures		
RR triangular index		0.053
TINN	(ms)	160.0

Poincare Plot*

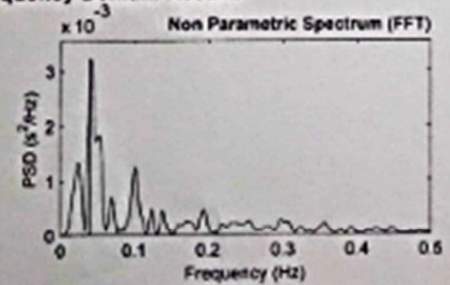
SD1 = 18.4 ms ↔ (Short-term HRV)
SD2 = 67.6 ms ↔ (Long-term HRV)



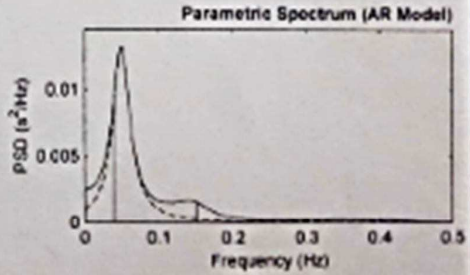
Distributions*



Frequency Domain Results



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF	0.0391	27	20.6	
LF	0.0410	70	52.8	66.3
HF	0.1934	36	26.8	33.7
LF/HF			1.964	



Frequency Band	Peak (Hz)	Power (ms^2)	Power (%)	Power (n.u.)
VLF	0.0000	0	0.0	
LF	0.0508	559	96.3	77.8
HF	0.3477	21	3.7	3.0
LF/HF			25.288	

25-Oct-2016 - HRV Analysis Software v1.1

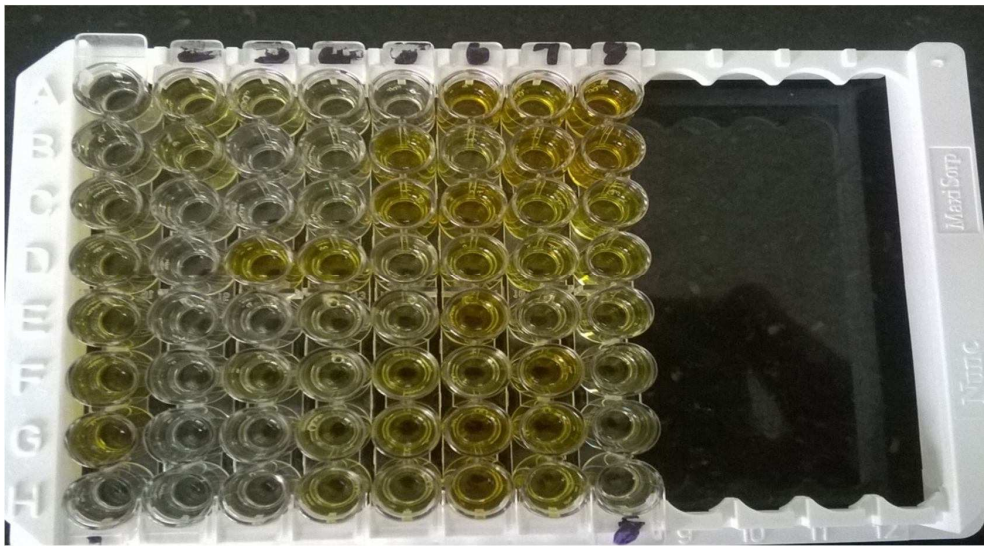
*Results are calculated from the non-detrended selected RRI signal.

The Biomedical Signal Analysis Group
Department of Applied Physics
University of Kuopio, Finland

1. SERUM SAMPLES FOR ANALYSIS

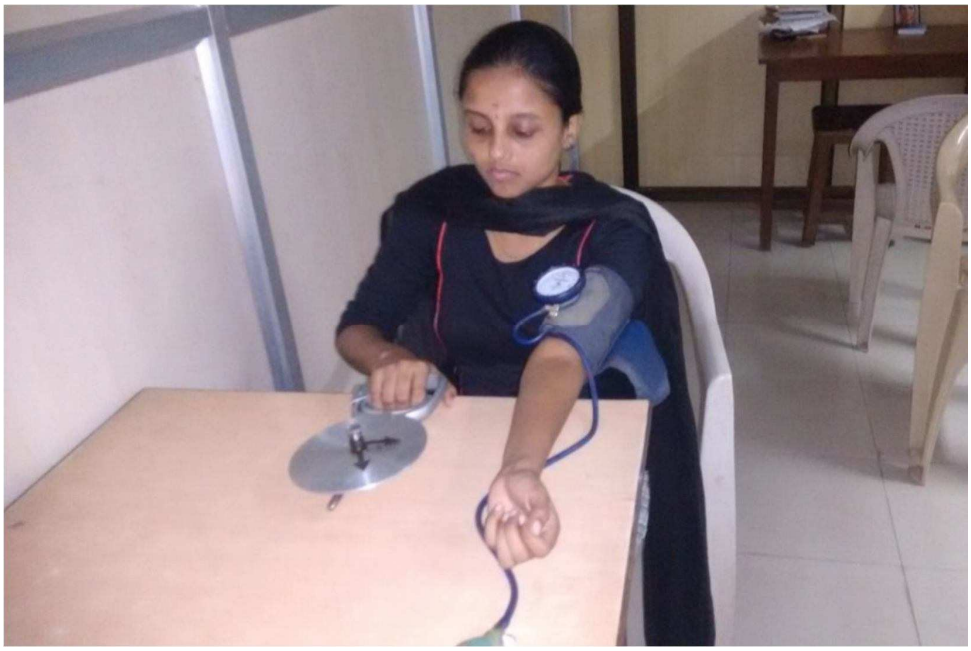
2. ELISA READER

3. DIACLONE ELISA KIT FOR SERUM IL 6



4. FINAL REACTION OF ELISA TEST FOR IL 6

5. RECORDING OF HRV



6. ISOMETRIC HAND GRIP TEST

MASTER CHART

S. No	AGE (yrs)	SEX	REST SBP (mmHg)	REST DBP (mmHg)	MEAN HR/min	nu LF	nu HF	LF/HF ratio	30/15 ratio	VR	E/I	OST SBP_1	IHGT DBP	IL 6 pg/ml	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TGL (mg/dl)	TC/HDL ratio	LDL/HDL ratio	BDI	SIS
1	26	F	128	84	76	38.6	61.4	0.63	1.11	1.26	1.11	-8	16	22	152	62	45	174	3.38	1.38	18	15
2	18	F	126	78	69	77.7	22.3	3.48	1.13	1.24	1.13	-4	12	25	139	59	40	163	3.48	1.48	11	16
3	16	F	126	78	69	48.8	51.2	0.95	1.08	1.12	1.06	6	20	1.5	159	75	42	190	3.79	1.79	13	15
4	22	M	130	80	76	34.9	65.1	0.54	1.14	1.21	1.15	-6	14	1	211	115	48	86	4.40	2.40	8	12
5	32	F	128	80	73	63.6	36.4	1.75	1.03	1.3	1.24	10	16	3	140	64	38	124	3.68	1.68	14	10
6	30	F	120	80	72	68.1	31.9	2.13	1.05	1.06	1.04	4	12	3	152	82	35	137	4.34	2.34	16	9
7	20	F	128	86	76	36.4	63.6	0.57	1.1	1.18	1.1	8	16	<1	205	109	48	149	4.27	2.27	8	13
8	27	F	124	82	79	21	79	0.27	1.13	1.22	1.09	-2	16	1	192	98	47	152	4.09	2.09	12	11
9	32	M	128	86	73	78.5	20.5	3.83	1.02	1.25	1.2	16	12	15	140	76	32	166	4.38	2.38	33	26
10	47	M	128	84	79	28.5	71.5	0.40	1.01	1.02	1.09	-6	18	<1	144	78	33	182	4.36	2.36	18	8
11	51	M	128	86	81	20.8	70.2	0.30	1.03	1.01	1.08	2	12	<1	168	94	37	176	4.54	2.54	18	12
12	24	F	124	86	69	66.1	33.9	1.95	0.9	1.31	1.14	12	14	180	129	73	28	155	4.61	2.61	31	27
13	39	F	128	80	84	41.8	58.2	0.72	1.12	1.1	1.18	6	20	<1	214	120	47	143	4.55	2.55	20	9
14	30	M	126	82	69	70.5	29.5	2.39	1.16	1.26	1.12	-4	10	13	117	57	30	173	3.90	1.90	31	25
15	31	M	138	80	70	52.2	47.8	1.09	1.1	1.14	1.01	-2	12	3	131	63	34	180	3.85	1.85	14	22
16	41	M	120	86	71	33.3	66.7	0.50	1.15	1.12	1.06	2	14	2.5	182	84	49	98	3.71	1.71	11	19
17	35	M	132	86	76	23.3	76.7	0.30	1.13	1.3	1.08	-4	18	7	168	92	38	124	4.42	2.42	21	20
18	50	F	128	80	83	70.7	29.3	2.41	1.12	1.06	1.12	-6	12	6	144	78	33	119	4.36	2.36	20	24
19	41	M	130	80	76	54.5	45.5	1.20	1.03	1.51	1.09	4	14	6	146	88	29	153	5.03	3.03	22	18
20	39	F	128	86	72	71.5	28.5	2.51	1.01	1.23	1.08	8	20	140	125	65	30	96	4.17	2.17	25	28
21	55	M	132	80	81	62.1	37.9	1.64	1.08	1.17	1.04	-4	16	9	170	90	40	148	4.25	2.25	16	22
22	36	F	130	80	70	53.1	46.9	1.13	1.06	1.15	1.06	-2	18	11	186	102	42	139	4.43	2.43	20	20
23	48	M	130	80	75	60.1	39.9	1.51	1.1	1.07	1.1	4	16	20	157	85	36	180	4.36	2.36	16	17
24	46	M	128	82	78	52.4	47.6	1.10	1.13	1.04	1.14	-4	18	28	151	79	36	175	4.19	2.19	19	15
25	47	M	130	82	65	43.9	56.1	0.78	1.06	1.01	1.18	2	14	3	151	63	44	153	3.43	1.43	13	13
26	33	F	132	80	74	80.8	19.2	4.21	1.07	1.43	1.07	-4	18	214	132	68	32	171	4.13	2.13	35	30
27	39	F	130	84	72	62.8	37.2	1.69	1.1	1.08	1.04	8	12	206	137	77	30	160	4.57	2.57	28	29
28	25	M	124	80	79	53.1	42.7	1.24	0.8	1.24	1.06	-4	12	11	152	64	44	126	3.45	1.45	15	14

S. No	AGE (yrs)	SEX	REST SBP (mmHg)	REST DBP (mmHg)	MEAN HR/min	nu LF	nu HF	LF/HF ratio	30/15 ratio	VR	E/I	OST SBP_1	IHGT DBP	IL 6 pg/ml	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TGL (mg/dl)	TC/HDL ratio	LDL/HDL ratio	BDI	SIS
29	40	M	126	80	69	65.1	42.7	1.52	1.12	1.09	1.05	4	16	10	179	107	36	143	4.97	2.97	14	11
30	37	F	136	80	74	67	33	2.03	0.9	1.3	1.18	2	20	79	185	105	40	149	4.63	2.63	29	18
31	39	F	124	80	72	71.4	28.6	2.50	1.11	1.04	1.03	8	12	86	139	69	35	170	3.97	1.97	32	21
32	45	M	130	82	78	72.4	27.6	2.62	1.13	1.07	1.1	6	8	22	153	95	29	166	5.28	3.28	28	21
33	28	F	130	80	74	45.5	54.5	0.83	1.08	1.45	1.16	10	12	203	158	92	33	154	4.79	2.79	30	30
34	21	M	124	80	73	37.8	62.2	0.61	1.14	1.06	1.11	-6	10	14	110	58	26	160	4.23	2.23	9	16
35	33	F	140	80	76	74.5	25.5	2.92	1.03	1.32	1.12	12	6	220	162	82	40	149	4.05	2.05	33	34
36	49	M	120	80	78	43.6	56.4	0.77	1.05	1.22	1.07	4	8	77	171	107	32	130	5.34	3.34	27	29
37	41	M	120	70	72	72.5	27.5	2.64	1.1	1.2	1.14	-2	20	223	120	58	31	170	3.87	1.87	28	29
38	43	M	128	78	80	56.4	43.6	1.29	1.13	1.1	1.01	-2	16	31	177	83	47	168	3.77	1.77	20	25
39	29	F	134	80	72	73.4	26.6	2.76	1.02	1.34	1.24	18	20	242	182	92	45	155	4.04	2.04	30	25
40	32	F	130	84	78	69.4	30.6	2.27	1.01	1.3	1.21	10	10	263	138	78	30	177	4.60	2.60	34	27
41	46	F	130	80	71	67.8	32.2	2.11	1.03	1.09	1.09	6	8	193	159	75	42	180	3.79	1.79	29	23
42	25	F	124	76	68	52.6	47.4	1.11	0.9	1.05	1.08	4	14	229	168	106	31	93	5.42	3.42	28	26
43	38	F	130	80	73	48.6	51.4	0.95	1.12	1.01	1.2	-2	14	66	184	112	36	96	5.11	3.11	27	22
44	52	M	126	82	76	57.2	42.8	1.34	1.16	1.1	1.31	-4	6	49	149	81	34	117	4.38	2.38	34	22
45	43	M	132	80	77	34.9	65.1	0.54	1.1	1.04	1.01	-4	12	12	169	89	40	110	4.23	2.23	26	19
46	27	F	130	86	73	62.8	37.2	1.69	1.15	1.09	1.07	-6	14	245	138	56	41	144	3.37	1.37	30	27
47	33	M	120	82	74	69	31	2.23	1.13	1.3	1.07	12	10	221	137	75	31	160	4.42	2.42	27	26
48	21	M	128	80	76	56.5	43.5	1.30	1.12	1.06	1.1	2	12	60	142	66	38	118	3.74	1.74	16	17
49	36	M	128	80	72	68.4	31.6	2.16	1.03	1.03	1.05	4	16	215	186	102	42	88	4.43	2.43	32	23
50	43	F	124	84	78	70.4	29.6	2.38	1.06	1.22	1.08	8	18	229	127	63	32	155	3.97	1.97	33	25
51	48	F	132	80	72	64.3	35.7	1.80	1.08	1.3	1.18	-2	16	72	140	74	33	143	4.24	2.24	29	22